
**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)*

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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 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, $\rho_{20} = 1,42$ 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 acid¹⁾ (H_2SO_4), concentrated, $\rho_{20} = 1,84$ g/ml.

4.7 Potassium sulfate¹⁾, 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 peroxide¹⁾ (H_2O_2), solution, $\rho_{20} = 1,099$ 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 acetate¹⁾, 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_2Cl) 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 ($\text{C}_{24}\text{H}_{16}\text{N}_2$)] 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 $[(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}]$ 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 $(20 \pm 2) ^\circ\text{C}$ and mix carefully. If, in the case of milk, the fat is not evenly dispersed, heat the sample slowly to $40 ^\circ\text{C}$, mix gently by inversion only, and cool quickly to $(20 \pm 2) ^\circ\text{C}$.

7.2 Buttermilk

If necessary, remove butter granules. Bring the sample to $(20 \pm 2) ^\circ\text{C}$ and mix carefully, immediately before weighing (see 8.1.1).

7.3 Plain yogurt and skimmed yogurt

Bring the sample to $(20 \pm 2) ^\circ\text{C}$ and mix carefully. If serum separates, stir vigorously, immediately before weighing (see 8.1.1).

7.4 Cream

Bring the sample to $(20 \pm 2) ^\circ\text{C}$. 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 $40 ^\circ\text{C}$ to facilitate mixing.

Cool the sample quickly to $(20 \pm 2) ^\circ\text{C}$. 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 $40 ^\circ\text{C}$ to $60 ^\circ\text{C}$. Remove and shake the container vigorously every 15 min. After 2 h, remove the container and cool to $(20 \pm 2) ^\circ\text{C}$. 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 $30 ^\circ\text{C}$ to $40 ^\circ\text{C}$. Cool to $(20 \pm 2) ^\circ\text{C}$. 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.

7.7 Dried whole milk, dried skimmed milk, dried whey, and dried buttermilk

Transfer the sample to a container of capacity about twice the volume of the sample and provided with an airtight lid. Close the container immediately. Mix the sample thoroughly by repeatedly shaking and inverting the container.

7.8 Butter

Because of the possible inhomogeneous distribution of iron in butter, the iron is determined in the serum. The iron content of the fat fraction, separated from the butter in the manner specified in this International Standard, is negligible compared with that of the serum and can be ignored.

Weigh, to the nearest 100 mg, 100 g of the sample into a dry previously tared centrifuge tube (5.2). Place the tube in a water bath maintained at $(45 \pm 1) ^\circ\text{C}$. As soon as the butter has melted, centrifuge the tube with a radial acceleration of 2 500g. Remove as much as possible of the clear fat layer by means of a pipette. Extract with 10 ml of light petroleum (4.4) and remove the upper layer by means of a pipette. Repeat these two operations twice. Remove residual light petroleum by warming in a water bath at $(65 \pm 1) ^\circ\text{C}$. Dry the outside of the tube with a clean paper tissue. Cool to $(20 \pm 2) ^\circ\text{C}$. Weigh the tube with its contents to the nearest 100 mg. Mix the contents carefully, immediately before weighing the test sample (see 8.1.5).

7.9 Anhydrous butterfat, butteroil, butterfat and ghee

Bring the sample to $40 ^\circ\text{C}$, maintain at this temperature for 5 min and mix gently. Cool to $(20 \pm 2) ^\circ\text{C}$.

7.10 Ice cream

For samples in small packages, remove the packaging and place the sample in a container provided with an airtight lid.

For samples taken from bulk or from large packages, keep them in their sample containers. In either case, melt the sample by standing the closed sample container in a water bath at $(45 \pm 1) ^\circ\text{C}$ for just sufficient time to allow the sample to become fluid. Mix the sample by shaking. Cool to $(20 \pm 2) ^\circ\text{C}$, continuing to mix until cooling is completed.

7.11 Cheese and processed cheese

Remove the rind, smear or mouldy surface layer of the cheese, in such a way as to provide a sample representative of the cheese as it is usually consumed. Grind the sample using the appropriate grinding device (5.3). Quickly mix the whole mass and, preferably, quickly grind the mass again. If the sample cannot be ground, mix the whole sample thoroughly.

Immediately transfer the pretreated sample, or a representative part of it, into a container provided with an airtight lid. Analyse the sample as soon as possible after grinding. Ground cheese showing unwanted mould growth or beginning to deteriorate shall not be examined.

7.12 Caseins, caseinates and coprecipitates

7.12.1 If most of the sample is sufficiently fine to pass through the sieve (5.4), it may be used without any grinding.

Transfer about 50 g of the sample as received into a container of capacity about twice the volume of the powder and provided with an airtight lid. Close the container immediately and mix the sample thoroughly by repeatedly shaking and inverting the container.

7.12.2 If most of the sample is not sufficiently fine to pass through the sieve (5.4), grind about 50 g of the sample until most of it does so. Transfer all the material into a container. Continue as specified in 7.12.1.

8 Procedure

WARNING — The method demands experience in trace analysis and careful execution. During the procedure, special consideration should be given to the problem of contamination, which will affect the accuracy and repeatability of the method.

8.1 Weighing and pretreatment of test sample

8.1.1 Milk, skimmed milk, whey, buttermilk, and yogurt

Weigh, to the nearest 10 mg, 10 g of the test sample into a digestion flask (5.7). Add 3 ml of nitric acid (4.5) and 1,8 ml of potassium sulfate solution (4.7). Continue as specified in 8.2.

8.1.2 Cream

Weigh, to the nearest 10 mg, 10 g of the test sample into a digestion flask (5.7). Add 8 ml of nitric acid (4.5). Heat the flask in a water bath at 80 °C to 90 °C for 1 h.

Shake vigorously every 3 min in order to wash the fat with the nitric acid. Cool to 40 °C and remove as much of the fat layer as possible by means of a pipette.

Add 15 ml of the light petroleum (4.4), swirl carefully, and remove the solvent by means of a pipette. Repeat twice with fresh 15 ml portions of the light petroleum. Remove residual light petroleum by warming in a water bath at 65 °C. Cool to room temperature. Add 1,8 ml of potassium sulfate solution (4.7). Continue as specified in 8.2.

8.1.3 Evaporated milk and sweetened condensed milk

Weigh, to the nearest 1 mg, 2,5 g of the test sample into a digestion flask (5.7). Add 4 ml of water (4.1), 3 ml of nitric acid (4.5) and 1,8 ml of potassium sulfate solution (4.7). Continue as specified in 8.2.

8.1.4 Dried whole milk, dried skimmed milk, dried whey and dried buttermilk

Weigh, to the nearest 1 mg, 1 g of the test sample into a digestion flask (5.7). Add 4 ml of water (4.1), and mix well. Then add 3 ml of nitric acid (4.5) and 1,8 ml of potassium sulfate solution (4.7). Continue as specified in 8.2.

8.1.5 Butter

Weigh, to the nearest 1 mg, 2 g of the butter serum (see 7.8) into a digestion flask (5.7). Add 3 ml of nitric acid (4.5) and 1,8 ml of potassium sulfate solution (4.7). Continue as specified in 8.2.

8.1.6 Anhydrous butterfat, butteroil, butterfat and ghee

Weigh, to the nearest 10 mg, 20 g of the fluid test sample into a digestion flask (5.7). Add 4 ml of water (4.1) and 8 ml of nitric acid (4.5).

Heat the flask in a water bath at 80 °C to 90 °C for 1 h. Shake thoroughly every 3 min in order to wash the fat with the nitric acid. Cool to 40 °C and remove as much of the fat layer as possible by means of a pipette.

Add 15 ml of the light petroleum (4.4), swirl carefully, and remove the solvent by means of a pipette. Repeat twice with fresh 15 ml portions of the light petroleum. Remove residual light petroleum by warming in a water bath at 65 °C. Cool to room temperature. Add 1,8 ml of potassium sulfate solution (4.7). Continue as specified in 8.2.

8.1.7 Ice cream

Weigh, to the nearest 1 mg, 2,5 g of the test sample into a digestion flask (5.7). Add 3 ml of nitric acid (4.5) and 1,8 ml of potassium sulfate solution (4.7). Continue as specified in 8.2.

8.1.8 Cheese and processed cheese

Weigh, to the nearest 1 mg, 1 g of the test sample into a digestion flask (5.7). Add 4 ml of water (4.1), 3 ml of nitric acid (4.5) and 1,8 ml of potassium sulfate solution (4.7). Continue as specified in 8.2.

8.1.9 Caseins, caseinates, and coprecipitates

Weigh, to the nearest 0,1 mg, in the case of caseins and caseinates, 0,75 g, and in the case of coprecipitates, 0,35 g, of the sample into a digestion flask (5.7). Add 4 ml of water (4.1), 3 ml of nitric acid (4.5) and 1,8 ml of potassium sulfate solution (4.7). Continue as specified in 8.2.

8.2 Determination

8.2.1 Digestion

8.2.1.1 Add three glass (quartz) beads (5.8) to the test portion in the digestion flask (5.7). Operating under a well-ventilated fume hood, place the flask in an inclined position and heat with a micro-burner (5.6). Control the height of the flame so as to limit the production of foam in the flask. Foaming into the neck of the flask is allowed, but the foam shall not escape. Keep the mixture simmering and avoid local overheating.

8.2.1.2 When the solution has turned brown, carefully add 3 to 5 drops of nitric acid (4.5). Heat vigorously as soon as possible. Continue heating and adding nitric acid, 5 to 20 drops at a time, swirling the flask occasionally to remove any material adhering to the wall, until the mixture remains colourless. Cool to room temperature.

8.2.1.3 Carefully add 2 ml of water (4.1) and 1 ml of hydrogen peroxide solution (4.8). Swirl and heat again until white fumes are emitted. Prevent loss through evaporation by allowing the sulfuric acid fumes to reflux in the neck of the flask. If the solution becomes yellow, cool to room temperature. Add a further 0,5 ml of hydrogen peroxide solution and then heat until white fumes are emitted. Continue heating for 45 min after the beginning of the emission of white fumes. Cool to room temperature and carefully add water to give a total volume of approximately 20 ml.

8.2.1.4 Add one or two drops of potassium permanganate solution (4.13) until the digest becomes faintly purple. Then add 3 ml of hydroxylammonium chloride solution (4.10) and mix well. Add 20 ml of sodium acetate solution (4.9) and about 15 ml of water (4.1). Mix well and allow to cool to room temperature. Make up to the 50 ml mark with water.

8.2.2 Colour development

Add, by means of a pipette, 4 ml of the bathophenanthroline solution (4.12) to the contents of the digestion flask (see 8.2.1.4) and close the flask with a stopper. Shake the flasks vigorously for 3 min ensuring that the stopper remains in position.

Cool under running tap water for at least 10 min and carefully tilt the flask several times after cooling. Keep the flask in a water bath at $(25 \pm 1) ^\circ\text{C}$ for 1 h.

8.3 Blank test

Carry out the blank test simultaneously with the determination.

Carry out a blank test using all the reagents used for the determination but replacing the test portion by 10 ml of water (4.1). During the digestion period, use the same amount of nitric acid (4.5) and hydrogen peroxide solution (4.8) as for the digestion of the test portion.

The absorbance of the blank test solution should correspond to less than 0,5 µg of iron. If the absorbance of the blank test corresponds to more than 0,5 µg of iron, all reagents should be checked.

It is recommended that the difference between two blank values be kept as low as possible (generally, this difference should not exceed 0,004 absorbance units).

8.4 Spectrometric measurements

Transfer the isoamyl alcohol (upper) layer, by means of a pipette, into a 10 mm pathlength cell (5.12). Measure the absorbances of the isoamyl alcohol layers of the test solution (see 8.2) and the blank test solution (see 8.3) at a wavelength of 533 nm against water (4.1) as reference. Subtract the value for the blank test solution from that of the test solution.

8.5 Number of determinations

Carry out all determinations, including the blank test (see 8.3), in duplicate.

8.6 Calibration graph

8.6.1 Pipette (5.11) 0 ml (zero member), 1 ml, 2 ml, 3 ml, 5 ml, and 10 ml of the iron standard solution (4.15) into a series of six digestion flasks (5.7). Dilute with water (4.1) to about 10 ml. Add to each flask 3 ml of nitric acid (4.5) and 1,8 ml of potassium sulfate solution (4.7).

8.6.2 Carry out the digestion as specified in 8.2.1 and the colour development as specified in 8.2.2.

8.6.3 Transfer each isoamyl alcohol (upper) layer, by means of a pipette, into a 10 mm pathlength cell (5.12). Measure the absorbance of each isoamyl alcohol layer at 533 nm against water (4.1) as reference. Subtract the value for the zero member from the values obtained for the other solutions.

8.6.4 Plot these absorbances against the amounts of iron contained in the calibration solutions.

8.6.5 Check the calibration graph weekly.

8.7 Performance of the method

It is recommended that the performance of the method be checked by analysing milk powder (or another milk product) of known or certified iron content.

9 Calculation and expression of results

9.1 Calculation

9.1.1 Products other than butter

The iron content, w_{Fe} , expressed in milligrams per kilogram, is given by the equation:

$$w_{Fe} = \frac{m_1}{m_0}$$

where

m_0 is the mass, in grams, of the test portion;

m_1 is the mass, in micrograms, of iron, read from the calibration graph (or calculated from the regression line obtained by the method of least squares).

9.1.2 Butter

Calculate the iron content of the butter serum as described in 9.1.1.

The iron content, w'_{Fe} , expressed in milligrams per kilogram, of butter is given by the equation:

$$w'_{Fe} = \frac{m_3}{m_2} w_{Fe}$$

where

m_2 is the mass, in grams, of the butter transferred to the centrifuge tube (see 7.8);

m_3 is the mass, in grams, of butter serum obtained in 7.8;

w_{Fe} is the iron content, in milligrams per kilogram, of the butter serum, calculated as specified in 9.1.1.

9.2 Expression of results

Take as the result the arithmetic mean of the results obtained, provided that the requirement for repeatability (see Clause 10) is satisfied.

Express the result to the number of decimal places shown in Table 1.

10 Repeatability

The difference between the results of duplicate determinations (results obtained almost simultaneously or in rapid succession by the same analyst) will not be greater than the repeatability value for the product analysed, as given in Table 1.

Table 1 — Expression of results and repeatability

Product	Expression of result to the nearest	Repeatability
	mg/kg	mg/kg
Milk	0,001	0,02
Skimmed milk	0,001	0,02
Whey	0,001	0,02
Buttermilk	0,001	0,03
Yogurt	0,001	0,03
Evaporated milk	0,01	0,1
Sweetened condensed milk	0,01	0,1
Dried whole milk	0,01	0,2
Dried skimmed milk	0,01	0,2
Dried whey, dried buttermilk	0,01	0,2
Cream	0,001	0,02
Butter	0,001	0,03
Anhydrous butterfat, butteroil, butterfat and ghee	0,001	0,005
Ice cream	0,01	0,2
Cheese and processed cheese	0,01	0,2
Caseins, caseinates and coprecipitates	0,1	0,4