INTERNATIONAL STANDARD

ISO 12081

IDF 36

Second edition
2010-06-01

Milk — Determination of calcium content — Titrimetric method

Lait — Détermination de la teneur en calcium — Méthode titrimétrique

Reference numbers
ISO 12081:2010(E)
IDF 36:2010(E)

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Published in Switzerland

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 12081 | IDF 36 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 12081 | IDF 36 cancels and replaces the first edition (ISO 12081:1998), 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.

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ISO 12081 | IDF 36 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 12081 | IDF 36 cancels and replaces IDF 36A:1992, of which it constitutes a minor revision.

Milk — Determination of calcium content — Titrimetric method

1 Scope

This International Standard specifies a titrimetric method for the determination of the calcium content of milk and of milk reconstituted from evaporated, condensed or dried milk.

2 Terms and definitions

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

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

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

3 Principle

The protein substances in a test portion are precipitated by trichloroacetic acid, then filtered. The calcium in the filtrate is precipitated as calcium oxalate and is separated by centrifuging. The washed and dissolved precipitate is titrated with potassium permanganate.

4 Reagents and materials

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

4.1 Trichloroacetic acid solution I ($C_2HCl_3O_2$), 200 g/l.

4.2 Trichloroacetic acid solution II, 120 g/l.

4.3 Ammonium oxalate ($C_2H_8N_2O_4$), saturated solution, cold.

4.4 Methyl red solution.

Dissolve 0,05 g of methyl red ($C_{15}H_{15}N_3O_2$) in 100 ml of ethanol (96 % volume fraction).

4.5 Acetic acid solution ($C_2H_4O_2$), 20 % volume fraction.

4.6 Ammonia solution I.

Mix equal volumes of ammonia (NH_3) solution (25 % mass fraction) and water.

4.7 Ammonia solution II.

Dilute 2 ml of ammonia solution (25 % mass fraction) with water to 100 ml.

4.8 Sulfuric acid (H_2SO_4).

Add 20 ml of sulfuric acid (98 % mass fraction) to 80 ml of water.

4.9 Potassium permanganate standard volumetric solution, $c(KMnO_4)$ = 0,004 mol/l ± 0,000 1 mol/l.

Check the titre by normal laboratory procedure using oxalic acid or sodium oxalate.

5 Apparatus and materials

Usual laboratory equipment and in particular the following.

5.1 Analytical balance, capable of weighing to the nearest 0,01 g, with a readability of 0,001 g.

5.2 One-mark volumetric flask, capacity 50 ml, ISO 1042[4] class A.

5.3 Pipette, capacity 20 ml, ISO 648[2] class A.

5.4 Centrifuge, capable of producing a radial acceleration of 1 400g.

5.5 Centrifuge tubes, cylindrical and round bottomed, capacity about 30 ml, graduated at 20 ml.

5.6 Pipettes, capacities 2 ml and 5 ml, ISO 648[2] class A.

5.7 Suction device, with capillary tube.

5.8 Water bath, capable of maintaining water at boiling point.

5.9 Burette, graduated in 0,02 ml divisions, ISO 385[1] class A.

5.10 Filter paper, ashless, for slow filtration.

6 Sampling

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

Bring the test sample of milk or reconstituted milk to a temperature of 20 °C ± 2 °C and mix carefully. If a homogeneous dispersion of the fat is not obtained, heat the sample slowly to 40 °C, then mix gently by repeated inversion and cool to 20 °C ± 2 °C.

8 Procedure

8.1 Test portion

Transfer approximately 20 g of the prepared test sample (see Clause 7) to the volumetric flask (5.2), using the pipette (5.3). Weigh the sample to the nearest 0,01 g.

8.2 Determination

8.2.1 Precipitation of protein substances

Gradually add, while shaking, trichloroacetic acid solution I (4.1) to the test portion (8.1) until a volume of 50 ml is obtained. Shake vigorously for a few seconds and allow to stand for 30 min. Filter through the filter paper (5.10), taking care that the filtrate obtained is clear.

8.2.2 Precipitation of calcium as oxalate and separation of the oxalate

Pipette (5.6) 5 ml of the clear filtrate (see 8.2.1), 5 ml of trichloroacetic acid solution II (4.2), 2 ml of ammonium oxalate solution (4.3), two drops of methyl red solution (4.4) and 2 ml of acetic acid solution (4.5) into a centrifuge tube (5.5). Mix by swirling.

Add ammonia solution I (4.6) drop by drop to the mixed solutions in the tube until the colour becomes pale yellow. Then add a few drops of acetic acid solution (4.5) until a pink coloration appears. Allow to stand for 4 h at room temperature.

Dilute the contents of the centrifuge tube with water to 20 ml. Centrifuge the tube at 1 400*g* for 10 min. Remove the clear supernatant liquid from the centrifuge tube with the suction device (5.7).

Rinse the walls of the centrifuge tube with 5 ml of ammonia solution II (4.7), taking care not to disturb the deposit of calcium oxalate. Centrifuge the tube at 1 400*g* again for 5 min. Remove the supernatant liquid from the centrifuge tube with the suction device (5.7).

Repeat this washing operation twice.

8.2.3 Titration

Add 2 ml of sulfuric acid (4.8) and 5 ml of water to the calcium oxalate deposit (see 8.2.2).

Place the tube in the boiling water bath (5.8) to dissolve the calcium oxalate deposit completely. Titrate the dissolved calcium oxalate with the potassium permanganate solution (4.9) until a pink colour persists. Take care that, during the titration, the temperature of the solution stays above 60 °C.

Record the volume, in millilitres, of potassium permanganate solution used, to the nearest 0,01 ml.

8.2.4 Blank test

Carry out a blank test in parallel with the determination by using 20 ml of water instead of the test portion.

Record the volume, in millilitres, of potassium permanganate solution used, to the nearest 0,01 ml.

9 Calculation and expression of results

9.1 Calculation

Calculate the calcium content, w_{Ca}, expressed as a percentage mass fraction, using the following equation:

$$w_{Ca} = 0{,}000\,4(V - V_0) \times \frac{1\,000 f}{m}$$

$$= 0{,}4\,(V - V_0) \times \frac{f}{m}$$

where

V is the volume, in millilitres, of potassium permanganate solution used for the test portion (see 8.2.3);

V_0 is the volume, in millilitres, of potassium permanganate solution used for the blank test (see 8.2.4);

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

f is the correction factor, given in Table 1, for the volume of precipitate resulting from the trichloroacetic acid precipitation.

Table 1 — Correction factor, f, as a function of the fat content of the sample

Fat content of the sample % mass fraction	Correction factor f
3,5 to 4,5	0,972
3	0,976
2	0,980
1	0,985
< 0,1	0,989

9.2 Expression of results

Express the results to three decimal places.

10 Repeatability

The absolute difference between two single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than 0,002 %.

11 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard (ISO 12081|IDF 36:2010);

d) any operating conditions not specified in this International Standard, or regarded as optional, as well as details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained;

f) if the repeatability has been checked, the final quoted result obtained.