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Milk — Determination of calcium content — Titrimetric method

Lait — Détermination de la teneur en calcium — Méthode titrimétrique

Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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.

International Standard ISO 12081 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with the International Dairy Federation (IDF) and the Association of Official Analytical Chemists (AOAC International), and will also be published by these organizations.

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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 Term and definition

For the purposes of this International Standard, the following term and definition apply.

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

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

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

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

4.1 Trichloroacetic acid solution I, 200 g/l.

4.2 Trichloroacetic acid solution II, 120 g/l.

4.3 Ammonium oxalate, saturated solution, cold.

4.4 Methyl red solution.

Dissolve 0,05 g of methyl red in 100 ml of ethanol (96 % volume fraction).

4.5 Acetic acid solution, 20 % volume fraction.

4.6 Ammonia solution I.

Mix equal volumes of ammonia 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.

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 ± 0,0001 mol/l.

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

5 Apparatus

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 Volumetric flask, of nominal capacity 50 ml.

5.3 Pipette, of nominal capacity 20 ml.

5.4 Centrifuge, capable of producing a radial acceleration of 1400 × g.

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

5.6 Pipettes, to deliver 2 ml and 5 ml.

5.7 Suction device, with capillary tube.

5.8 Water bath, capable of boiling water.

5.9 Burette, graduated in 0,02 ml.

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 [1].

It is important that the laboratory receive a sample which is truly representative and 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 (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

Add gradually the trichloroacetic acid solution I (4.1) to the test portion (8.1), while shaking, 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 ml of the clear filtrate (8.2.1), 5 ml of the trichloroacetic acid solution II (4.2), 2 ml of the ammonium oxalate solution (4.3), two drops of the methyl red solution (4.4) and 2 ml of the acetic acid solution (4.5) into a centrifuge tube (5.5). Mix by swirling.

Add the ammonia solution I (4.6) drop by drop to the mixed solutions in the centrifuge tube until the colour becomes pale yellow. Then add a few drops of the 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 centrifuge tube at $1400 \times g$ 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 the ammonia solution II (4.7). Take care not to disturb the deposit of calcium oxalate. Centrifuge the centrifuge tube at $1400 \times g$ again for 5 min. Remove the supernatant liquid from the centrifuge tube with the suction device.

Repeat this washing operation twice.

8.2.3 Titration

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

Place the tube in the boiling water bath 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 content of calcium using the following equation:

$$w = 0{,}0004\left(V - V_0\right) \times \frac{1000\,f}{m}$$

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

where

w is the content of calcium, expressed as a mass fraction in percent;

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

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

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

f is the correction factor for the volume of precipitate resulting from the trichloroacetic acid precipitation, as follows.

Fat content of the sample (%)	Correction factor
3,5 to 4,5	f = 0,972
3	f = 0,976
2	f = 0,980
1	f = 0,985
< 0,1	f = 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 specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, with reference to this International Standard;

— all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

— the test result(s) obtained; or

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

Bibliography

[1] ISO 707, *Milk and milk products — Guidance on sampling.*

ICS 67.100.10

Descriptors: agricultural products, food products, dairy products, milk, chemical analysis, determination of content, calcium, volumetric analysis.

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