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Milk — Determination of total phosphorus content — Method using molecular absorption spectrometry

Lait — Détermination de la teneur en phosphore total — Méthode par spectrométrie d'absorption moléculaire

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Foreword

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ISO 9874|IDF 42 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 edition of ISO 9874|IDF 42 cancels and replaces ISO 9874:1992, of which it constitutes a minor revision.

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has 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.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the IDF National Committees casting a vote.

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ISO 9874 | IDF 42 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/AOAC Group of Experts on *Nitrate, nitrite and phosphorus in cheese and other dairy products* (E8), under the aegis of its chairman, Mr. G. Bråthen (NO).

This edition of ISO 9874 | IDF 42 cancels and replaces IDF 42B:1992.

Milk — Determination of total phosphorus content — Method using molecular absorption spectrometry

1 Scope

This International Standard specifies a molecular absorption spectrometric method for the determination of the total phosphorus content of milk.

2 Normative references

The following referenced documents are indispensable for the application 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 648:1977, *Laboratory glassware — One-mark pipettes*

ISO 1042:1998, *Laboratory glassware — One-mark volumetric flasks*

ISO 4788:2005, *Laboratory glassware — Graduated measuring cylinders*

3 Terms and definitions

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

3.1
total phosphorus content
mass fraction of substances determined by the method specified in this International Standard

NOTE It is expressed as a mass fraction in percent.

4 Principle

A sample of milk is treated by a wet digestion method using sulfuric acid and hydrogen peroxide, or by dry ashing. Molybdenum blue is formed by the addition of molybdate/ascorbic acid solution. The absorbance at a wavelength of 820 nm is measured spectrophotometrically.

5 Reagents

All reagents shall be of recognized analytical grade, unless otherwise specified. The water used shall be distilled or deionized water, free from phosphorus compounds.

5.1 Concentrated sulfuric acid, ρ_{20} = 1,84 g/ml, $c(H_2SO_4) \approx 18$ mol/l.

5.2 Dilute sulfuric acid, $c(H_2SO_4) \approx 5$ mol/l.

Carefully add, while stirring continuously, 278 ml of concentrated sulfuric acid (5.1) to 722 ml of water.

5.3 Dilute hydrochloric acid, c(HCl) ≈ 1 mol/l. (Used for dry ashing.)

Dilute 83 ml of concentrated hydrochloric acid (ρ_{20} = 1,19 g/ml) to 1 000 ml with water.

5.4 Hydrogen peroxide, $c(H_2O_2)$ ≈ 9 mol/l, free from phosphorus-containing substances.

5.5 Sodium molybdate solution, $c(Na_2MoO_4)$ ≈ 0,1 mol/l.

Weigh 2,5 g of sodium molybdate dihydrate into a 100 ml one-mark volumetric flask (6.10). Add a sufficient volume of dilute sulfuric acid (5.2) to dissolve the sodium molybdate dihydrate. Dilute to the mark with the same sulfuric acid (5.2) and mix.

5.6 Ascorbic acid solution, $c(C_6H_8O_6)$ ≈ 0,25 mol/l.

Weigh 5 g of ascorbic acid into a 100 ml one-mark volumetric flask (6.10). Add a sufficient volume of water to dissolve the ascorbic acid. Dilute to the mark with water and mix.

This solution shall be freshly prepared.

5.7 Molybdate/ascorbic acid solution.

Immediately before use, add 25 ml of the sodium molybdate solution (5.5) to 10 ml of the ascorbic acid solution (5.6) in a 100 ml one-mark volumetric flask (6.10). Dilute to the mark with water and mix.

5.8 Standard solution A.

Dry about 1 g of potassium dihydrogen orthophosphate (KH_2PO_4) for at least 48 h in a desiccator (6.14). Weigh 0,439 4 g of the dried phosphate into a 1 000 ml one-mark volumetric flask (6.10). Dilute to the mark with water and mix.

The phosphorus content of this standard solution is 100 mg/l.

5.9 Standard solution B.

Pipette 10 ml of the standard solution A (5.8) into a 100 ml one-mark volumetric flask (6.10). Dilute to the mark with water and mix well.

The phosphorus content of this standard solution is 10 mg/l.

6 Apparatus

IMPORTANT — All glassware shall be thoroughly cleaned using a phosphorus-free detergent and then rinsed with water.

Usual laboratory equipment and, in particular, the following.

6.1 Analytical balance, accurate to the nearest 0,1 mg.

6.2 Water bath, capable of operating at 100 °C.

6.3 Oven, capable of operating at 100 °C.

6.4 Electric heater or **micro gas burner**.

6.5 Digestion flask (Kjeldahl) or **test tubes**, of 50 ml capacity.

6.6 Glass beads, of approximately 5 mm diameter.

6.7 Dish, made of platinum or silica, of approximately 55 mm diameter, and a suitable **watch-glass**.

6.8 Electric furnace with air circulation, capable of operating at 500 °C to 550 °C.

6.9 Graduated cylinders, of 5 ml and 25 ml capacity, in accordance with the requirements of ISO 4788.

6.10 One-mark volumetric flasks, of 50 ml, 100 ml and 1 000 ml capacity, in accordance with the requirements of ISO 1042:1998, class B.

6.11 One-mark pipettes, delivering 1 ml, 2 ml, 3 ml, 5 ml and 10 ml, in accordance with the requirements of ISO 648:1997, class B.

6.12 Molecular absorption spectrometer, suitable for measurements at a wavelength of 820 nm, equipped with cells of 10 mm optical path length.

6.13 Filter paper, medium grade.

6.14 Desiccator, containing an efficient drying agent.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707 | IDF 50 [1].

8 Preparation of test sample

Bring the laboratory sample to 20 °C ± 2 °C and mix carefully. If a homogeneous dispersion of the fat is not obtained, heat the sample slowly to 40 °C, mix gently and cool to 20 °C ± 2 °C, before taking a test sample for analysis.

9 Procedure

9.1 Wet-digestion method

9.1.1 Weigh into a digestion flask (6.5), to the nearest 1 mg, a test portion of about 1,5 g of the test sample (Clause 8). Add three glass beads (6.6) and 4 ml of the concentrated sulfuric acid (5.1).

9.1.2 Operating under a well-ventilated fume hood provided with a water scrubbing system, place the flask in an inclined position and heat using the electric heater or micro gas burner (6.4). Control the heating so as to limit the production of foam in the flask.

Keep the mixture boiling gently. Avoid local overheating and avoid heating the flask above the surface of the liquid contents.

9.1.3 As soon as the foaming stops, cool the mixture in air to room temperature. Carefully add 2 ml of the hydrogen peroxide (5.4) and reheat. Repeat this procedure until the contents have become clear and colourless. During heating, mix the contents from time to time by swirling carefully. Avoid local overheating.

9.1.4 Cool the mixture in air to room temperature and rinse the neck of the flask with about 2 ml of water. Heat the contents again until the water has evaporated. Allow the liquid to boil for 30 min in order to destroy all traces of hydrogen peroxide. Avoid local overheating.

9.1.5 Cool the mixture in air to room temperature. Quantitatively transfer the liquid contents into a 100 ml one-mark volumetric flask (6.10). Dilute to the mark with water and mix well.

9.1.6 Pipette 2 ml of the test solution into a 50 ml one-mark volumetric flask (6.10) and dilute with about 25 ml of water. Add 2,0 ml of the molybdate/ascorbic acid solution (5.7). Dilute to the mark with water and mix well.

9.1.7 Boil the contents of the flask in the water bath (6.2) for 15 min.

9.1.8 Cool the mixture to room temperature in cold water. Proceed as specified in 9.5.

The spectrometric determination should be carried out within 1 h.

9.2 Dry-ashing method

9.2.1 Weigh, to the nearest 1 mg, a test portion of about 10 g of the test sample (Clause 8) into a platinum or silica dish (6.7).

9.2.2 Evaporate to dryness in an oven (6.3) set at 100 °C or lower, or on the water bath (6.2).

9.2.3 After drying is complete, heat the test sample in the electric furnace (6.8) at a temperature between 500 °C and 550 °C until white (or nearly white) ash is obtained.

The dish should preferably be heated on a hotplate to burn off ignitable contents before being placed in the furnace.

9.2.4 Allow the dish and contents to cool in the furnace and then cover with a watch-glass. Dissolve the ash in 2 ml or 3 ml of the dilute hydrochloric acid (5.3) and dilute with about 3 ml of water.

9.2.5 Quantitatively transfer the solution of ash to a 100 ml one-mark volumetric flask (6.10). Rinse the watch-glass and dish with water and transfer the washings to the flask. Dilute to the mark with water and mix well. Filter through a medium-grade filter paper (6.13).

9.2.6 Pipette 10 ml of the filtrate into a 100 ml one-mark volumetric flask (6.10). Dilute to the mark with water and mix well.

9.2.7 Pipette 2 ml of the test solution into a 50 ml one-mark volumetric flask (6.10) and dilute with about 25 ml of water. Add 2,0 ml of the molybdate/ascorbic acid solution (5.7). Dilute to the mark with water and mix well.

9.2.8 Boil the contents of the flask in the water bath (6.2) for 15 min.

9.2.9 Cool the mixture to room temperature in cold water. Proceed as specified in 9.5.

The spectrometric determination should be carried out within 1 h.

9.3 Blank test

Carry out a blank test concurrently with the determination, using the same procedure as for the test portion (9.1 or 9.2) but using 1,5 ml or 10 ml, respectively, of phosphorus-free water in place of the test portion.

9.4 Calibration graph

9.4.1 Pipette, into a series of five 50 ml one-mark volumetric flasks (6.10), 0 ml, 1 ml, 2 ml, 3 ml and 5 ml, respectively, of the standard solution B (5.9). Dilute the contents of each flask to approximately 25 ml with water.

9.4.2 Add to the contents of each volumetric flask, 2,0 ml of the molybdate/ascorbic acid solution (5.7). Dilute each solution to the mark with water and mix well. The resulting solutions contain 0 µg, 10 µg, 20 µg, 30 µg, and 50 µg of phosphorus, respectively, per 50 ml.

9.4.3 Boil the contents of the flasks in the water bath (6.2) for 15 min.

9.4.4 Cool the solutions to room temperature in cold water. Within 1 h, measure the absorbance of each of the calibration solutions against that of the solution containing 0 µg of phosphorus (see 9.4.2) using the spectrometer (6.12) at a wavelength of 820 nm, with cells of 10 mm optical path length.

If the absorbance value of the solution containing 0 µg of phosphorus per 50 ml is high, check the reagents.

9.4.5 Plot the net absorbance values obtained against the mass, in micrograms, of phosphorus contained in the calibration solutions (9.4.2).

9.5 Spectrometric measurement

Within 1 h, carry out the spectrometric measurements on the test solution (9.1.8 or 9.2.9) against the blank (9.3) using the spectrometer (6.12) at a wavelength of 820 nm with cells of 10 mm optical path length.

10 Expression of results

Using the calibration graph (9.4), determine the mass of phosphorus corresponding to the net absorbance value of the test solution. Calculate the total phosphorus content w_P, expressed as a mass fraction in percent, using the following formula.

a) Wet-digestion method

$$w_P = \frac{m_1}{200\ m_0}$$

b) Dry-ashing method

$$w_P = \frac{m_1}{20\ m_0}$$

where

m_0 is the mass of the test portion (9.1.1 or 9.2.1), in grams;

m_1 is the mass of phosphorus, read or calculated from the calibration graph, in micrograms.

Report the result to the third decimal place.

11 Precision

11.1 Interlaboratory test

The precision of the method has been established by an international interlaboratory test (see Reference [2] in the Bibliography) carried out in accordance with ISO 5725 [3]. The values obtained for repeatability and reproducibility are expressed at the 95 % probability level.

11.2 Repeatability

The absolute difference between two independent 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 0,005 % (mass fraction).

Reject both results if the difference exceeds 0,005 % (mass fraction) and carry out two new single determinations.

11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than 0,016 % (mass fraction).

NOTE For definitions of repeatability and reproducibility, see ISO 5725-1 [4].

12 Test report

The test report shall specify:

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;

d) 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);

e) the test result(s) obtained, or, if the repeatability has been checked, the final quoted result obtained.

Bibliography

[1] ISO 707 | IDF 50, *Milk and milk products — Guidance on sampling*

[2] *Bulletin of the International Dairy Federation*, 1986, No. 207

[3] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests* (now withdrawn)

[4] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

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