

Cheese and processed cheese products — Determination of total phosphorus content — Molecular absorption spectrometric method

ICS 67.100.30

National foreword

This British Standard is the UK implementation of ISO 2962:2010. It supersedes BS 770-6:1984 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/5, Chemical analysis of milk and milk products.

A list of organizations represented on this committee can be obtained on request to its secretary.

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*Fromages et fromages fondus — Détermination de la teneur en
phosphore total — Méthode par spectrométrie d'absorption moléculaire*



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Foreword

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ISO 2962|IDF 33 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 third edition of ISO 2962|IDF 33 cancels and replaces the second edition (ISO 2962:1984), 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 2962|IDF 33 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 2962|IDF 33 cancels and replaces IDF 33C:1987, of which it constitutes a minor revision.

Cheese and processed cheese products — Determination of total phosphorus content — Molecular absorption spectrometric method

1 Scope

This International Standard specifies a molecular absorption spectrometric method for the determination of the total phosphorus content of cheese. The method is applicable to all kinds of cheese and to processed cheese products.

2 Terms and definitions

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

2.1

total phosphorus content in cheese and processed cheese products

mass fraction of substances determined by the method specified in this International Standard

NOTE Total phosphorus content is expressed as a percentage mass fraction.

3 Principle

Cheese is digested by concentrated sulfuric acid and hydrogen peroxide.

Molybdenum blue is formed by addition of a sodium molybdate-ascorbic acid solution. Molecular absorption of the blue colour formed is measured spectrometrically at a wavelength of 820 nm.

NOTE Dry ashing can be used, provided that the procedure gives comparable results to the wet digestion.

4 Reagents

Unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity, free from phosphorus compounds.

4.1 Concentrated sulfuric acid (H_2SO_4), $\rho_{20} = 1,84$ g/ml.

4.2 Hydrogen peroxide, solution containing about 30 g of H_2O_2 per 100 ml.

4.3 Molybdate-ascorbic acid solution.

4.3.1 Sodium molybdate solution.

Dissolve 12,5 g of sodium molybdate dihydrate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$) in a 5 mol/l sulfuric acid solution, make up to 500 ml with this sulfuric acid solution and mix.

4.3.2 Ascorbic acid solution.

Dissolve 10 g of ascorbic acid ($C_6H_8O_6$) in water, make up to 200 ml, and mix.

This solution cannot be stored and shall be prepared immediately before use.

4.3.3 Mixed solution.

Immediately before use, mix 25 ml of sodium molybdate solution (4.3.1) with 10 ml of ascorbic acid solution (4.3.2), make up to 100 ml with water, and mix.

4.4 Phosphorus, standard solution corresponding to 100 µg of phosphorus per millilitre.

Dry, for at least 48 h, about 1 g of potassium dihydrogenorthophosphate (KH_2PO_4) in a desiccator over an efficient desiccant, e.g. concentrated sulfuric acid.

Dissolve 0,439 4 g of the previously dried phosphate in water, make up to 1 000 ml, and mix.

5 Apparatus

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

Usual laboratory equipment and in particular the following.

5.1 Analytical balance.

5.2 Device for grinding or grating cheese, capable of being easily cleaned.

5.3 Water bath.

5.4 Digestion flasks (Kjeldahl flasks or digestion tubes), capacity 25 ml.

5.5 Heating apparatus.

5.5.1 Micro gas burners or electric heaters, for heating Kjeldahl flasks.

5.5.2 Heating block, for heating digestion tubes.

5.6 Glass beads.

5.7 Measuring cylinders, capacities 5 ml and 25 ml, ISO 4788^[5] class A.

5.8 One-mark volumetric flasks, capacities 50 ml and 100 ml, ISO 1042^[4] class B.

5.9 Pipettes, capacities 1 ml, 2 ml, 3 ml, 5 ml and 10 ml, ISO 648^[1] class B or ISO 835^[3].

5.10 Spectrometer, suitable for measurements at a wavelength of 820 nm, equipped with cells of optical pathlength 10 mm.

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^[2].

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

Remove the rind, smear or mouldy surface layer of cheese, in such a way as to provide a sample representative of the cheese as it is usually consumed. Grind or grate the sample using an appropriate device (5.2). Mix the ground or grated mass quickly and, if possible, grind or grate a second time and again mix thoroughly. If the sample cannot be ground or grated, mix it thoroughly by intensive stirring and kneading.

Transfer the test sample to an airtight container to await analysis, which should be carried out as soon as possible after grinding or grating. If delay is unavoidable, take all precautions to ensure proper preservation of the sample and to prevent condensation of moisture on the inside surface of the container.

Clean the device after grinding or grating the sample.

8 Procedure

8.1 Test portion

Into a digestion flask (5.4), weigh, to the nearest 1 mg, a test portion of 0,5 g to 1,0 g of the test sample.

If the water content of the cheese is less than 50 % mass fraction, a test portion of approximately 0,5 g is sufficient. In the case of fresh cheese, a test portion of approximately 1,0 g can be taken.

8.2 Determination

8.2.1 Add three glass beads and 4 ml of the concentrated sulfuric acid (4.1) in a digestion flask and heat under a well-ventilated fume hood. A Kjeldahl flask shall be placed in an inclined position.

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.

Maintain the mixture at simmering point. Avoid local overheating and avoid heating the flask above the surface of the liquid contents.

8.2.2 As soon as the foaming stops, cool to room temperature. Carefully add a few drops of the hydrogen peroxide solution (4.2), reheat, and repeat this procedure until the contents have become clear and colourless. During heating, mix the contents from time to time by careful swirling. Avoid local overheating.

8.2.3 Rinse the neck of the flask with about 2 ml of water, and heat the contents again until the water has evaporated.

Allow the liquid to boil for 30 min after colour disappearance in order to destroy traces of hydrogen peroxide. Avoid local overheating.

8.2.4 Cool to room temperature. Quantitatively transfer the liquid contents into a 100 ml one-mark volumetric flask (5.8). Make up to the mark with water and mix well.

8.2.5 Pipette (5.9) 1 ml of the solution into a 50 ml one-mark volumetric flask (5.8) and dilute with about 25 ml of water. Add 20 ml of the molybdate-ascorbic acid solution (4.3.3). Make up to the mark with water and mix well.

8.2.6 Heat the flask in a boiling water bath (5.3) for 15 min.

8.2.7 Cool to room temperature in a cold water bath (5.3). Within 1 h, measure the absorbance of the solution against that of the blank test solution (see 8.4) at a wavelength of 820 nm.

8.3 Calibration curve

8.3.1 Pipette (5.9) 10 ml of the phosphorus standard solution (4.4) into a 100 ml one-mark volumetric flask (5.8). Make up to the mark with water and mix well.

8.3.2 Pipette into a series of five 50 ml one-mark volumetric flasks (5.8) 0 ml, 1 ml, 2 ml, 3 ml, and 5 ml, respectively, of the diluted standard solution (8.3.1), i.e. equivalent to 0 µg, 10 µg, 20 µg, 30 µg, and 50 µg of elemental phosphorus, respectively. Dilute the contents of each flask to approximately 20 ml with water.

8.3.3 Add 20 ml of the molybdate-ascorbic acid solution (4.3.3) to the contents of each volumetric flask. Make up to the mark with water and mix well.

Proceed as specified in 8.2.6.

8.3.4 Cool to room temperature in cold water. Within 1 h, measure the absorbance of each of the calibration solutions against water as reference at a wavelength of 820 nm.

8.3.5 Plot these absorbances against the amounts of elemental phosphorus added.

8.4 Blank test

Carry out a blank test by following the procedure specified in 8.2, but without a test portion.

9 Calculation

The total phosphorus content, w_P , expressed as a percentage by mass, is given by the equation

$$w_P = \frac{m_1}{100 m_0}$$

where

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

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

Report the result to two places of decimals.

10 Precision

10.1 Repeatability

The difference between two single results obtained on identical test material by one analyst using the same apparatus within a short time interval will exceed 0,03 g of phosphorus per 100 g of product on average not more than once in 20 cases in the normal and correct operation of the method.

10.2 Reproducibility

The difference between two single and independent results obtained by two operators working in different laboratories on identical test material will exceed 0,06 g of phosphorus per 100 g of product on average not more than once in 20 cases in the normal and correct operation of the method.

11 Test report

The test report shall contain at least the following information:

- a) all the information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method used, including a reference to this International Standard (ISO 2962|IDF 33:2010);
- d) any operating conditions not specified in this International Standard, or regarded as optional, as well as details of any incidents that may have influenced the result(s);
- e) the test result(s) obtained;
- f) if the repeatability has been checked, the final quoted result obtained.

Bibliography

- [1] ISO 648, *Laboratory glassware — Single-volume pipettes*
- [2] ISO 707|IDF 50, *Milk and milk products — Guidance on sampling*
- [3] ISO 835, *Laboratory glassware — Graduated pipettes*
- [4] ISO 1042, *Laboratory glassware — One-mark volumetric flasks*
- [5] ISO 4788, *Laboratory glassware — Graduated measuring cylinders*

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