INTERNATIONAL STANDARD ISO 2962

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Cheese and processed cheese products — Determination of phosphorus content (Reference method)

Fromages et fromages fondus — Détermination de la teneur en phosphore (Méthode de référence)

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FOREWORD

ISO (the International Organization for Standardization) is a worldwide federation of national standards institutes (ISO Member Bodies). The work of developing International Standards is carried out through ISO Technical Committees. Every Member Body interested in a subject for which a Technical Committee has been set up has the right to be represented on that Committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work.

Draft International Standards adopted by the Technical Committees are circulated to the Member Bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 2962 was drawn up by Technical Committee ISO/TC 34, *Agricultural food products*, and circulated to the Member Bodies in September 1972.

It has been approved by the Member Bodies of the following countries :

Australia	Germany	Poland
Austria	Hungary	Romania
Belgium	India	South Africa, Rep. of
Brazil	Iran	Thailand
Czechoslovakia	Ireland	Turkey
Egypt, Arab Rep. of	Israel	United Kingdom
Finland	Netherlands	
France	New Zealand	

This International Standard has also been approved by the International Union of Pure and Applied Chemistry (IUPAC).

No Member Body expressed disapproval of the document.

NOTE – This International Standard has been developed jointly with the IDF (International Dairy Federation) and the AOAC (Association of Official Analytical Chemists, U.S.A.) on the basis of an IDF Standard for the purpose of being included in the FAO/WHO Code of Principles concerning Milk and Milk Products and Associated Standards.

The text as approved by the above organizations was also published by FAO/WHO (Code of Principles, Standard No. B-12), by the IDF (IDF Standard No. 33A) and by the AOAC (Official Methods of Analysis).

Printed in Switzerland

Cheese and processed cheese products — Determination of phosphorus content (Reference method)

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a reference method for the determination of the phosphorus content of cheese and processed cheese products.

2 REFERENCE

ISO/R 707, *Milk and milk products — Sampling.*

3 DEFINITION

phosphorus content of cheese and processed cheese products : The percentage by mass of phosphorus determined by the procedure specified.

4 PRINCIPLE

Digestion of the cheese with concentrated sulphuric acid in the presence of hydrogen peroxide. Formation of molybdenum blue by treatment of the phosphate obtained with sodium molybdate and hydrazine sulphate as a reducing agent and photometric determination of the phosphorus content.

5 REAGENTS

All reagents used shall be of analytical reagent quality and the water used shall be distilled water or water of at least equivalent purity.

5.1 Sulphuric acid, concentrated (ρ_{20} 1,84 g/ml).

5.2 Hydrogen peroxide 30 % (*m/m*) solution.

5.3 Sodium molybdate-hydrazine sulphate reagent :

5.3.1 Sodium molybdate 25 g/l solution in 10 N sulphuric acid.

Dissolve 12,5 g of sodium molybdate ($Na_2MoO_4.2H_2O$) in 10 N sulphuric acid to a volume of 500 ml.

5.3.2 Hydrazine sulphate 1,5 g/l solution

Dissolve 0,30 g of hydrazine sulphate ($H_2NNH_2.H_2SO_4$) in water to a volume of 200 ml.

5.3.3 Mix, immediately before use, 25 ml of solution 5.3.1 with 10 ml of solution 5.3.2 and dilute this mixture to 100 ml with water. This solution cannot be stored.

5.4 Phosphate standard solution

Dissolve in water 0,439 0 g of potassium dihydrogen orthophosphate (KH_2PO_4) dried beforehand for 48 h over an efficient drying agent, for example concentrated sulphuric acid, and dilute to a volume of 1 000 ml. This solution contains 100 μg of phosphorus in 1 ml.

Dilute 10 ml of the standard solution with water to a volume of 100 ml.

6 APPARATUS

6.1 Analytical balance.

6.2 Photoelectric colorimeter or **spectrophotometer,** suitable for making readings at a wavelength of 700 nm.

6.3 Suitable grinding device.

6.4 Kjeldahl flasks, capacity 25 ml.

6.5 Digestion apparatus to hold the Kjeldahl flasks in an inclined position, and with a heating device which will not heat the part of the flask above the surface of the liquid contents.

6.6 Boiling aids for digestion : broken porcelain or glass beads.

6.7 Volumetric flasks, of 50, 100, 200, 500 and 1 000 ml, complying with ISO/R 1042.

6.8 Pipettes and/or **burettes,** to deliver 1, 2, 5, 10, 20 and 25 ml complying with ISO/R 648 and ISO/R 385.

7 SAMPLING

See ISO/R 707.

8 PROCEDURE

8.1 Preparation of the test sample

Before the analysis, remove the rind or mouldy surface layer of the cheese so as to give a test sample representative of the cheese as it is usually consumed. Grind or treat the

sample so obtained in such a way that it will be homogeneous; avoid losses by evaporation. Keep the test sample so prepared in an airtight container until analysis, which shall be carried out on the same day.

8.2 Test portion

Weigh, to the nearest 0,001 g, about 0,5 g of the test sample.

8.3 Digestion

8.3.1 Place successively into a Kjeldahl flask (6.4) the test portion, a few boiling aids (6.6) and 4 ml of sulphuric acid (5.1).

8.3.2 Heat the Kjeldahl flask carefully on the digestion apparatus (6.5). As soon as the foaming stops, cool to room temperature. Carefully add some drops of the hydrogen peroxide solution (5.2), reheat, and repeat this procedure until the contents have become clear and colourless. During the heating, mix the contents from time to time. Avoid local overheating.

8.3.3 Rinse the neck of the flask with about 2 ml of water, then heat the contents again until the water has been evaporated.

8.3.4 Allow the liquid to boil for half an hour after it has become clear, in order to destroy traces of hydrogen peroxide. Avoid local overheating.

8.3.5 After cooling to room temperature, add approximately 20 ml of water, mix, and after cooling again, transfer the liquid contents into a 100 ml volumetric flask (6.7); fill to the mark with water and mix.

8.4 Determination

8.4.1 Pipette 1 ml of the solution into a 50 ml volumetric flask (6.7) and dilute with about 25 ml of water. Add 20 ml of the sodium molybdate-hydrazine sulphate reagent (5.3), fill to the mark with water and mix.

8.4.2 Place the flask in boiling water and allow the colour to develop for 15 min.

8.4.3 Cool to room temperature in cold water and measure the absorbance within 1 h against the blank (8.6) at a wavelength of 700 nm.

8.4.4 Carry out two determinations on the same test sample.

8.5 Preparation of the calibration curve

8.5.1 Place in five 50 ml volumetric flasks (6.7), 0, 1, 2, 5 and 10 ml respectively of the diluted standard solution (5.4) to provide a suitable range of standards containing 0 (zero value), 10, 20, 50 and 100 μg of phosphorus.

8.5.2 Add water to the flasks to make a volume of about 25 ml; add 20 ml of the sodium molybdate-hydrazine sulphate reagent, dilute to the mark with water, mix and proceed as described in 8.4.2 and 8.4.3, measuring the absorbance of the standards against the zero value.

8.5.3 Prepare the calibration curve by plotting the absorbance against the quantity of phosphorus, in micrograms.

8.6 Blank test

Carry out a blank test following the same procedure but without the test portion.

9 EXPRESSION OF RESULTS

9.1 Method of calculation and formula

9.1.1 Convert the reading obtained as directed in 8.4.3 to micrograms of phosphorus by reference to the calibration curve.

9.1.2 The phosphorus content of the sample, as a percentage by mass, is equal to

$$\frac{m_1}{100\, m_0}$$

where

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

m_1 is the mass of phosphorus, in micrograms, as obtained in 9.1.1.

9.2 Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst shall not exceed 0,04 g of phosphorus per 100 g of the product.

10 TEST REPORT

The test report shall show the method used and the result obtained. It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that may have influenced the result.

The report shall include all details required for the complete identification of the sample.