International Standard 4099

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION•МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ•ORGANISATION INTERNATIONALE DE NORMALISATION

Cheese — Determination of nitrate and nitrite contents — Method by cadmium reduction and photometry

Fromages — Détermination des teneurs en nitrates et en nitrites — Méthode par réduction au cadmium et photométrie

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Every member body interested in a subject for which a technical committee has been established 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. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 4099 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

ISO 4099 was first published in 1978. This second edition cancels and replaces the first edition, of which it constitutes a minor revision.

NOTE — The method specified in this International Standard has been developed jointly with the IDF (International Dairy Federation) and the AOAC (Association of Official Analytical Chemists, USA). The text as approved by the above organizations will also be published by FAO/WHO (Code of Principles concerning Milk and Milk Products and Associated Standards), by the IDF and by the AOAC (Official Methods of Analysis).

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Cheese — Determination of nitrate and nitrite contents — Method by cadmium reduction and photometry

1 Scope and field of application

This International Standard specifies a method by cadmium reduction and photometry for the determination of the nitrate and nitrite contents of cheese.

The method is suitable for hard, semi-hard and soft cheeses of various ages and for processed cheese.

The detection limits of the method are 5 mg of nitrate per kilogram and 0,5 mg of nitrite per kilogram.

2 Reference

ISO 707, *Milk and milk products — Methods of sampling.*

3 Definition

nitrate and nitrite contents of cheese : The contents of substances determined by the procedure specified in this International Standard and expressed respectively as milligrams of nitrate ion (NO_3^-) and of nitrite ion (NO_2^-) per kilogram.

4 Principle

Extraction of the cheese with warm water, precipitation of the fat and proteins, and filtration.

Reduction of the nitrate in a portion of the filtrate to nitrite, by means of copperized cadmium.

Development of a red colour, in portions of both unreduced filtrate and of the reduced solution, by addition of sulfanilamide and *N*-1-naphthyl-ethylenediamine dihydrochloride, and spectrometric measurement at a wavelength of 538 nm.

Calculation of the nitrite content of the sample and of the total nitrite content after reduction of nitrate, by comparing the measured absorbances with those of a series of standard sodium nitrite solutions; calculation of the nitrate content from the difference between these two contents.

5 Reagents

All reagents shall be of recognized analytical grade. The water used shall be distilled or deionized water, free from nitrate and nitrite.

NOTE — In order to avoid possible inclusion of small gas bubbles in the copperized cadmium column (6.10), the distilled or deionized water used for the preparation of the column (8.1), for checking the reducing capacity of the column (8.2), and for regeneration of the column (8.3) should preferably be freshly boiled and afterwards cooled to room temperature.

5.1 Cadmium, granules, diameter 0,3 to 0,8 mm.

If cadmium granules are not available commercially, they may be prepared as follows.

Place a suitable number of zinc rods in a beaker and cover with a 40 g/l solution of cadmium sulfate octahydrate ($CdSO_4 \cdot 8H_2O$). From time to time, scrape the cadmium sponge from the rods over a period of 24 h. Remove the zinc rods and decant the liquid until only sufficient remains to cover the cadmium. Wash the sponge two or three times with water. Transfer the cadmium to a laboratory blender together with 400 ml of 0,1 mol/l hydrochloric acid, and blend for a few seconds to obtain granules of the required size. Return the contents of the blender to the beaker and leave to stand for several hours, occasionally stirring to remove bubbles. Decant most of the liquid and immediately copperize as described in 8.1.1 to 8.1.5.

5.2 Copper(II) sulfate, solution.

Dissolve 20 g of copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in water and dilute to 1 000 ml.

5.3 Buffer solution, pH 9,6 to 9,7.

Dilute 50 ml of concentrated hydrochloric acid [ϱ_{20} 1,19 g/ml; about 38 % (m/m) hydrogen chloride solution] with 600 ml of water. After mixing, add 135 ml of ammonium hydroxide [ϱ_{20} 0,91 g/ml; about 25 % (m/m) ammonia solution]. Dilute to 1 000 ml with water and mix.

NOTE — If ammonium hydroxide of this concentration is not available, an equivalent amount of a more concentrated solution may be used, for example 100 ml of 35 % (*m*/*m*) solution (ϱ_{20} 0,88 g/ml).

Adjust the pH to 9,6 to 9,7 if necessary.

5.4 Hydrochloric acid, about 2 mol/l.

Dilute 160 ml of concentrated hydrochloric acid (ϱ_{20} 1,19 g/ml) to 1 000 ml with water.

5.5 Hydrochloric acid, about 0,1 mol/l.

Dilute 50 ml of the hydrochloric acid (5.4) to 1 000 ml with water.

5.6 Solutions for precipitation of proteins and fat.

5.6.1 Zinc sulfate, solution.

Dissolve 53,5 g of zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) in water and dilute to 100 ml.

5.6.2 Potassium hexacyanoferrate(II), solution.

Dissolve 17,2 g of potassium hexacyanoferrate(II) trihydrate [$K_4Fe(CN)_6 \cdot 3H_2O$] in water and dilute to 100 ml.

5.7 EDTA, solution.

Dissolve 33,5 g of disodium ethylenediaminetetraacetate dihydrate ($Na_2C_{10}H_{14}N_2O_8 \cdot 2H_2O$) in water and dilute to 1 000 ml.

5.8 Solutions for colour development.

5.8.1 Solution I.

Dissolve, by heating on a water-bath, 0,5 g of sulfanilamide ($NH_2C_6H_4SO_2NH_2$) in a mixture of 75 ml of water and 5 ml of concentrated hydrochloric acid (ϱ_{20} 1,19 g/ml). Cool to room temperature and dilute to 100 ml with water. Filter if necessary.

5.8.2 Solution II.

Dilute 450 ml of concentrated hydrochloric acid (ϱ_{20} 1,19 g/ml) to 1 000 ml with water.

5.8.3 Solution III.

Dissolve 0,1 g of *N*-1-naphthyl-ethylenediamine dihydrochloride ($C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$) in water. Dilute to 100 ml with water. Filter if necessary.

The solution may be stored for up to 1 week in a well-stoppered brown bottle in a refrigerator.

5.9 Sodium nitrite, standard solution corresponding to 0,001 g of nitrite per litre.

On the day of use, dissolve in water 0,150 g of sodium nitrite ($NaNO_2$), dried to constant mass at 110 to 120 °C, dilute to 1 000 ml with water in a one-mark volumetric flask and mix.

Dilute 10 ml of this solution with 20 ml of the buffer solution (5.3) and dilute further to 1 000 ml with water in a one-mark volumetric flask. Mix.

1 ml of this standard solution contains 1,00 µg of NO_2^-.

5.10 Potassium nitrate, standard solution corresponding to 0,004 5 g of nitrate per litre.

Dissolve in water 1,468 g of potassium nitrate (KNO_3), dried to constant mass at 110 to 120 °C, dilute to 1 000 ml with water in a one-mark volumetric flask and mix.

On the day of use, dilute 5 ml of this solution with 20 ml of the buffer solution (5.3) and dilute further to 1 000 ml with water in a one-mark volumetric flask. Mix.

1 ml of this standard solution contains 4,50 µg of NO_3^-.

6 Apparatus

All glassware shall be thoroughly cleaned and rinsed with distilled water to ensure that it is free from nitrate and nitrite.

Usual laboratory apparatus, and in particular

6.1 Analytical balance.

6.2 Appropriate grinding device.

6.3 Suitable laboratory mixer/homogenizer, with glass containers of capacity 250 or 400 ml.

6.4 Conical flasks, of capacity 250 ml.

6.5 Volumetric flasks, of capacities 100; 500 and 1 000 ml, complying with the requirements of ISO 1042, class B.

6.6 Pipettes, to deliver 2; 4; 5; 6; 8; 10; 12; 20 and 25 ml, complying with the requirements of ISO 648, class A, or ISO 835/1.

NOTE — Where appropriate, burettes may be used instead of pipettes.

6.7 Graduated cylinders, of capacities 5; 10; 25; 100; 250; 500 and 1 000 ml.

6.8 Glass funnels, of diameter about 7 cm, with short stem.

6.9 Filter paper, medium grade, of diameter about 15 cm, free from nitrate and nitrite.

6.10 Reduction column (for example as shown in the figure).

6.11 Spectrometer, suitable for measuring absorbance at a wavelength of 538 nm, with cells of optical path length 1 to 2 cm.

7 Sampling

7.1 See ISO 707.

7.2 Store the sample in such a way that deterioration and change in composition are prevented.

8 Procedure

8.1 Preparation of the copperized cadmium column

8.1.1 Transfer the cadmium granules (5.1) (approximately 40 to 60 g for each column) into a conical flask (6.4).

8.1.2 Add sufficient of the hydrochloric acid (5.4) to cover the cadmium. Swirl for a few minutes.

8.1.3 Decant the solution and wash the cadmium in the flask with water, until it is free from chloride.

8.1.4 Copperize the cadmium granules by adding the copper(II) sulfate solution (5.2) (about 2,5 ml per gram of cadmium) and swirling for 1 min.

8.1.5 Decant the solution and wash the copperized cadmium immediately with water, taking care that the cadmium is continuously covered with water. Terminate the washing when the wash water is free from precipitated copper.

8.1.6 Fit a glass wool plug to the bottom of the glass column intended to contain the copperized cadmium (see the figure). Fill the glass column with water.

8.1.7 Transfer the copperized cadmium into the glass column with minimum exposure to air. The height of the copperized cadmium should be 15 to 20 cm.

NOTES

1 Avoid trapping air bubbles between the copperized cadmium granules.

2 Take care that the level of the liquid does not fall below the top of the copperized cadmium.

8.1.8 Condition the newly prepared column by running through it a mixture of 750 ml of water, 225 ml of the standard potassium nitrate solution (5.10), 20 ml of the buffer solution (5.3) and 20 ml of the EDTA solution (5.7), at a flow rate not exceeding 6 ml/min, then wash the column with 50 ml of water.

8.2 Checking the reducing capacity of the column

Carry out this check at least twice a day, at the beginning and at the end of a series of determinations.

8.2.1 Pipette 20 ml of the standard potassium nitrate solution (5.10) into the reservoir on top of the column. Immediately add 5 ml of the buffer solution (5.3) to the contents of the reservoir. Collect the eluate in a 100 ml volumetric flask. The flow rate shall not exceed 6 ml/min.

8.2.2 When the reservoir has nearly run empty, wash the walls of the reservoir with about 15 ml of water and, when this has run off, repeat the same treatment with another 15 ml portion of water. After this second portion of water has run into the column as well, completely fill the reservoir with water and allow it to pass through the column at maximum flow rate.

8.2.3 After nearly 100 ml of eluate has been collected, remove the volumetric flask, make up to the mark with water and mix well.

8.2.4 Pipette 10 ml of the eluate into a 100 ml volumetric flask. Add water to obtain a volume of about 60 ml. Proceed as specified in 8.8.2, 8.8.3 and 8.8.4.

8.2.5 From the nitrite content (in micrograms of nitrite ion per millilitre) of the diluted eluate (8.2.4), determined from the calibration curve (8.10), calculate the percentage reducing capacity of the column (0,067 µg of NO_2^- per millilitre corresponds to 100 % reducing capacity). If the reducing capacity is less than 95 %, the column should be regenerated.

8.3 Regeneration of the column

Regenerate the column as follows, at the end of each day after use, or more frequently if the check (8.2) indicates a loss of efficiency.

8.3.1 Add about 5 ml of the EDTA solution (5.7) and 2 ml of the hydrochloric acid (5.5) to 100 ml of water. Run the mixture through the column at a flow rate of about 10 ml/min.

8.3.2 When the reservoir has run empty, wash the column with water, the hydrochloric acid (5.5) and water successively.

8.3.3 If the column still does not show a satisfactory efficiency, repeat the procedure specified in 8.1.8.

8.4 Preparation of the test sample

Prior to analysis, remove the rind or mouldy surface layer of the cheese, in such a way as to obtain a sample representative of the cheese as it is usually consumed. Grind the sample by means of an appropriate device; mix the ground mass quickly, and, if possible, grind it a second time and again mix thoroughly. Clean the device after grinding each sample. If the sample cannot be ground, mix it thoroughly by intensive kneading.

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

Ground cheese showing unwanted mould growth or beginning to deteriorate should not be examined.

8.5 Test portion

Weigh, to the nearest 0,01 g, approximately 10 g of the test sample and transfer it quantitatively into the glass container of the mixer/homogenizer (6.3).

8.6 Extraction and deproteination

8.6.1 Add gradually 144 ml of warm water (50 to 55 °C) to the test portion. Mix in the mixer/homogenizer until the cheese is well suspended.

8.6.2 Add, in the following order, 6 ml of the zinc sulfate solution (5.6.1), 6 ml of the potassium hexacyanoferrate(II) solution (5.6.2) and 40 ml of the buffer solution (5.3) to the cheese suspension, swirling thoroughly after each addition.

8.6.3 After 3 min, filter through a filter paper (6.9), collecting the filtrate in a 250 ml conical flask.

NOTE — It is essential that the filtrate is clear. For this purpose, if well-matured cheeses are analysed, it might be necessary to use a larger quantity of precipitation reagents. If this is the case, then the volume of warm water which is added in 8.6.1 should be diminished by the same quantity.

8.7 Reduction of nitrate to nitrite

8.7.1 Pipette 20 ml of the filtrate (8.6.3) into the reservoir on top of the reduction column. Add 5 ml of the buffer solution (5.3) to the contents of the reservoir. Collect the eluate in a 100 ml volumetric flask. The flow rate shall not exceed 6 ml/min.

8.7.2 When the reservoir has nearly run empty, wash the walls of the reservoir with about 15 ml of water and, when this has run off, repeat the same treatment with another 15 ml portion of water. After this second portion of water has run into the column as well, completely fill the reservoir with water and allow it to flow through the column at maximum flow rate.

8.7.3 After nearly 100 ml of eluate has been collected, remove the volumetric flask, make up to the mark with water and mix well.

8.8 Determination

8.8.1 For nitrite determination, pipette an adequate volume (between 5 and 40 ml) of the filtrate (8.6.3) into a 100 ml volumetric flask.

For nitrate determination, pipette an adequate volume (between 5 and 40 ml) of the eluate (8.7.3) into another 100 ml volumetric flask.

Add water to each to obtain a volume of about 60 ml. Then treat the contents of each flask as in 8.8.2, 8.8.3 and 8.8.4.

8.8.2 Add 6 ml of solution II (5.8.2) and then 5 ml of solution I (5.8.1). Mix carefully and leave the solution for 5 min at room temperature, protected from direct sunlight.

8.8.3 Add 2 ml of solution III (5.8.3). Mix carefully and leave the solution for 5 min at room temperature, protected from direct sunlight. Make up to the mark with water and mix well.

8.8.4 Measure within 15 min the absorbance of the solution against that of a reagents blank (8.9) at a wavelength of 538 nm.

8.9 Blank test

Carry out a reagents blank test using all the reagents, but omitting the test portion.

8.10 Calibration curve

8.10.1 Pipette 0; 2; 4; 8; 12 and 20 ml of the standard sodium nitrite solution (5.9) into separate 100 ml volumetric flasks. Add water to each volumetric flask to obtain volumes of about 60 ml.

8.10.2 Carry out the procedure described in 8.8.2 and 8.8.3.

8.10.3 Measure within 15 min the absorbances of the solutions against that of the first solution (containing no sodium nitrite) at a wavelength of 538 nm.

8.10.4 Plot the absorbances obtained in 8.10.3 against the nitrite concentrations, in micrograms per millilitre, calculated from the amounts of standard sodium nitrite solution added (see 8.10.1).

9 Expression of results

9.1 Nitrite content

9.1.1 Method of calculation and formula

The nitrite content of the sample, expressed as milligrams of nitrite ion (NO_2^-) per kilogram, is equal to

$$\frac{20\,000 \times \varrho_1}{m \times V}$$

where

ϱ_1 is the concentration, in micrograms of nitrite ion per millilitre, read from the calibration curve, corresponding to the measured absorbances (8.8.4) of the solution obtained using the filtrate (8.6.3);

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

V is the volume, in millilitres, of the aliquot taken (8.8.1) from the diluted filtrate (8.6.3).

Report the result to the nearest 0,1 mg/kg.

9.1.2 Repeatability

The difference between two results obtained within a short time interval by the same analyst should not exceed 1 mg/kg.

9.2 Nitrate content

9.2.1 Method of calculation and formula

The nitrate content of the sample, expressed as milligrams of nitrate ion (NO_3^-) per kilogram, is equal to

$$1{,}35\left[\frac{100\,000 \times \varrho_2}{m \times V} - w(NO_2^-)\right]$$

where

ϱ_2 is the concentration, in micrograms of nitrite ion per millilitre, read from the calibration curve, corresponding to the measured absorbance (8.8.4) of the solution obtained using the eluate (8.8.1);

$w(NO_2^-)$ is the nitrite content of the sample, expressed in milligrams per kilogram, calculated as described in 9.1.1;

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

V is the volume, in millilitres, of the aliquot taken (8.8.1) from the eluate (8.7.3).

NOTE — If the reducing capacity of the column is taken into account, the nitrate content of the sample, expressed as milligrams of nitrate ion per kilogram, is equal to

$$1{,}35\left[\frac{100\,000 \times \varrho_2}{m \times V} - w(NO_2^-)\right]\frac{100}{r}$$

where r is the reducing capacity of the column at the end of a series of determinations.

Report the result to the nearest 1 mg/kg.

9.2.2 Repeatability

The difference between two results obtained within a short time interval by the same analyst should not exceed 3 mg/kg if the nitrate content is less than 30 mg/kg, and should not exceed 10 % of the arithmetic mean of the results if the nitrate content is greater than or equal to 30 mg/kg.

9.2.3 Reproducibility

The difference between two single and independent results obtained by two operators working in different laboratories on identical test material should not exceed 6 mg/kg if the nitrate content is less than 30 mg/kg, and should not exceed 25 % of the arithmetic mean of the results if the nitrate content is greater than or equal to 30 mg/kg.

10 Test report

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

The test report shall include all the information necessary for the complete identification of the sample.

Dimensions in millimetres

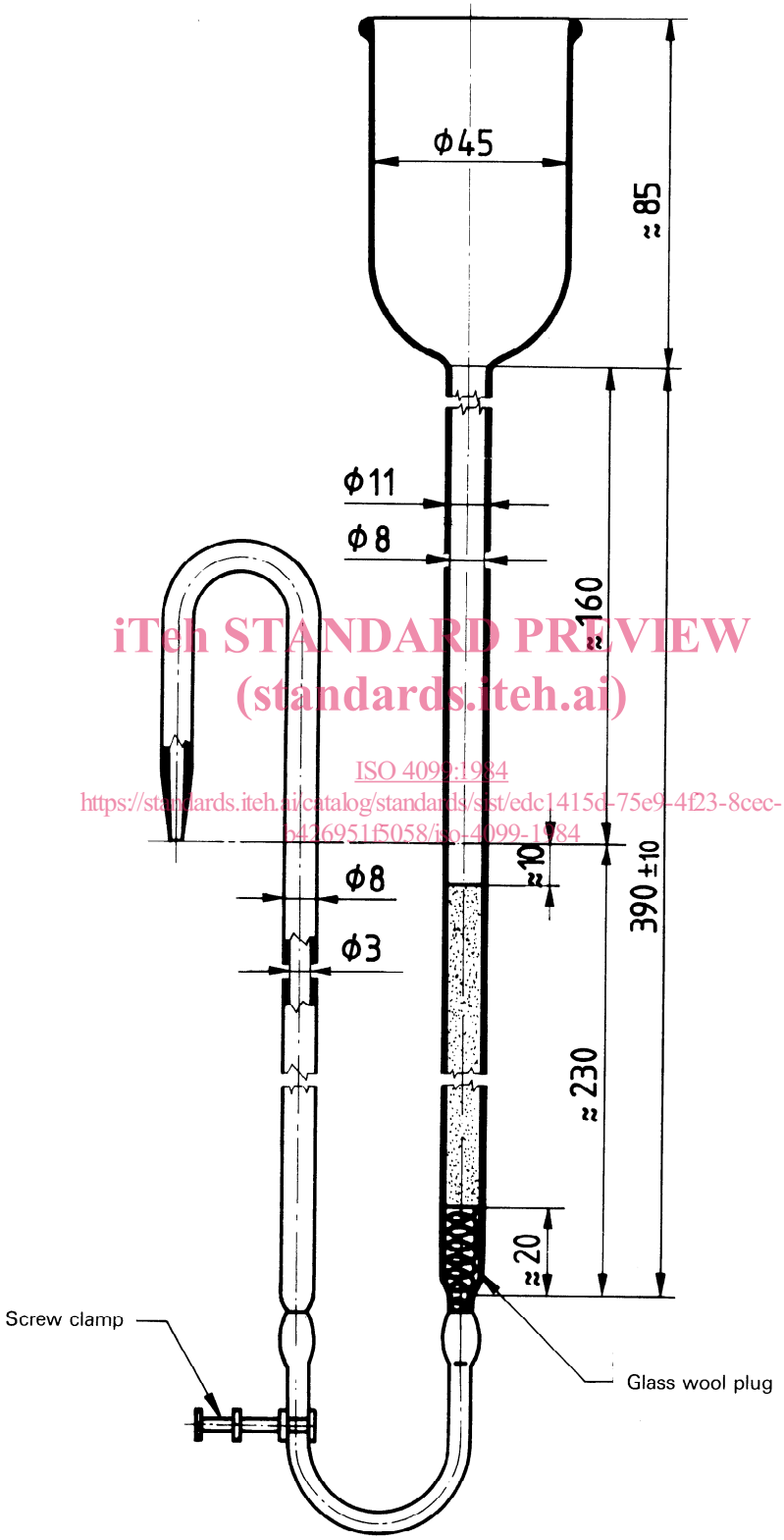

Figure — Apparatus for nitrate reduction