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

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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 4099 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in March 1977.

It has been approved by the member bodies of the following countries :

Australia	Ghana	Poland
Austria	Hungary	Portugal
Belgium	India	Romania
Bulgaria	Iran	South Africa, Rep. of
Canada	Ireland	Spain
Czechoslovakia	Israel	Switzerland
Egypt, Arab Rep. of	Korea, Rep. of	Thailand
Ethiopia	Mexico	Turkey
France	Netherlands	United Kingdom
Germany, F. R.	New Zealand	Yugoslavia

No member body expressed disapproval of the document.

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, U.S.A.). 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.

2 REFERENCE

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

3 DEFINITIONS

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 (parts per million).

4 PRINCIPLE

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

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

Development of a red colour, in portions of both unreduced filtrate and of the reduced solution, by addition of sulphanilamide and N-1-naphthyl-ethylenediamine dihydrochloride, and photometric 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 analytical quality. The water used shall be distilled or deionized, free from nitrite and nitrate.

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 sulphate. 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 distilled water. Transfer the cadmium to a laboratory blender together with 400 ml of 0,1 N hydrochloric acid solution, 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) sulphate solution.

Dissolve 20 g of copper(II) sulphate pentahydrate ($CuSO_4.5H_2O$) 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*) HCl] with 600 ml of water. After mixing, add 100 ml of concentrated ammonia solution [ρ_{20} 0,88 g/ml; about 35 % (*m/m*) NH_3]. Dilute to 1 000 ml with water and mix.

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

5.4 Hydrochloric acid solution, about 2 N.

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

5.5 Hydrochloric acid solution, about 0,1 N.

Dilute 50 ml of 2 N hydrochloric acid solution (5.4) to 1 000 ml with water.

5.6 Solutions for precipitation of proteins and fat.

5.6.1 Zinc sulphate solution.

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

5.6.2 Potassium hexacyanoferrate(II) solution.

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

5.7 EDTA solution.

Dissolve 33,5 g of disodium ethylenedinitrilotetraacetate (disodium ethylenediaminetetraacetate) dihydrate ($Na_2C_{10}H_{14}N_2O_8.2H_2O$) 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 sulphanilamide ($NH_2C_6H_4SO_2NH_2$) 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 ($C_{10}H_7NHCH_2CH_2NH_2.2HCl$) 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.

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.

On the day of use, 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 final dilution contains 1,00 µg of NO_2^-.

5.10 Potassium nitrate, standard solution.

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

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 final dilution 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.

6.1 Analytical balance.

6.2 Appropriate grinding device.

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

6.4 Conical flasks of 250 ml capacity.

6.5 Volumetric flasks of 100, 500 and 1 000 ml capacity, complying with ISO 1042, class B.

6.6 Pipettes, to deliver 2 — 4 — 5 — 6 — 8 — 10 — 12 — 20 — 25 and 50 ml, complying with ISO 648, class A, or ISO/R 835.

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

6.7 Graduated cylinders of 5 — 10 — 25 — 100 — 250 — 500 and 1 000 ml capacity.

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

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

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

6.11 Photoelectric colorimeter or **spectrophotometer,** suitable for making readings at a wavelength of 538 nm with cells of 1 to 2 cm optical path length.

7 SAMPLING

7.1 See ISO/R 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 2 N hydrochloric acid solution (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 copper(II) sulphate 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 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 not to allow the level of the liquid to 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 standard potassium nitrate solution (5.10), 20 ml of buffer solution (5.3) and 20 ml of 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 standard potassium nitrate solution (5.10) into the reservoir on top of the column. Immediately add 5 ml of 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 8.9.2, 8.9.3 and 8.9.4.

8.2.5 If the nitrite concentration of the diluted eluate (8.2.4), as determined from the calibration curve (8.10), is below 0,063 µg of NO_2^- per millilitre (i.e. 95 % of theoretical value), 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 EDTA solution (5.7) and 2 ml of 0,1 N hydrochloric acid solution (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, 0,1 N hydrochloric acid solution 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 smear or mouldy surface layer of the cheese, in such a way as to provide 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 a second time and again mix thoroughly. If the sample cannot be ground, mix it thoroughly by intensive stirring and kneading.

Transfer the test sample to an air-tight container to await analysis, which should be carried out as soon as possible after grinding. 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. Ground cheese showing unwanted mould growth of beginning to deteriorate should not be examined.

Clean the device after grinding each sample.

8.5 Test portion

Weigh 10 g of the test sample, to the nearest 1 mg, and transfer it quantitatively into the glass container of the mixer/homogenizer (6.3).

8.6 Extraction and deproteination

8.6.1 Add gradually 164 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 zinc sulphate solution (5.6.1), 6 ml of potassium hexacyanoferrate(II) solution (5.6.2) and 20 ml of 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 necessary to obtain a clear filtrate. 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 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 Preparation of solution for determination of nitrite in sample

Pipette 20 ml of the filtrate (8.6.3) into a 100 ml volumetric flask, make up to the mark with water and mix well.

8.9 Determination

8.9.1 Pipette equal aliquots (for example 25 ml) of the diluted filtrate (8.8) and of the eluate (8.7.3) into separate 100 ml volumetric flasks. Add water to each to obtain a volume of about 60 ml. Then treat the contents of each flask as in 8.9.2, 8.9.3 and 8.9.4.

8.9.2 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.

8.9.3 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.

8.9.4 Measure within 15 min the absorbance of the solution against that of a reagents blank (8.10) at a wavelength of 538 nm.

8.9.5 Carry out two determinations on the same diluted filtrate (8.8) and two determinations on the same eluate (8.7.3).

8.10 Blank test

Carry out a reagents blank test using all reagents and 4 ml of water instead of the test portion.

8.11 Calibration curve

8.11.1 Pipette 0 – 2 – 4 – 6 – 8 – 10 – 12 – 16 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.

8.11.2 Carry out the procedure described in 8.9.2 and 8.9.3.

8.11.3 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.

8.11.4 Plot the absorbances obtained in 8.11.3 against the nitrite concentrations, in micrograms per millilitre, calculated from the volumes of standard sodium nitrite solution added (see 8.11.1).

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

$$NO_2^- = \frac{100\,000 \times c_1}{m \times V}$$

where

c_1 is the concentration, in micrograms of NO_2^- per millilitre, read from the calibration curve, that corresponds with the measured absorbance (8.9.4) of the solution obtained using the diluted filtrate (8.8);

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

V is the volume, in millilitres, of the aliquot taken (8.9.1) from the diluted filtrate (8.8).

Take as the result the arithmetic mean of the two determinations (8.9.5).

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

9.1.2 *Repeatability*

The difference between the results of a determination in duplicate (results obtained almost simultaneously or in rapid succession by the same analyst) shall not exceed 1 mg/kg.