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Caseins and caseinates — Determination of nitrate and nitrite contents — Method by cadmium reduction and spectrometry

Caséines et caséinates — Détermination des teneurs en nitrates et en nitrites — Méthode par réduction au cadmium et spectrométrie

Reference number
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Foreword

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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 8195 was prepared by Technical Committee ISO/TC 34, *Agricultural food products.*

NOTE — The method specified in this International Standard has been developed jointly with the International Dairy Federation (IDF) and the Association of Official Analytical Chemists (AOAC) and will also be published by these organizations.

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

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Caseins and caseinates — Determination of nitrate and nitrite contents — Method by cadmium reduction and spectrometry

1 Scope and field of application

This International Standard specifies a method by cadmium reduction and spectrometry for the determination of the nitrate and nitrite contents of caseins and caseinates.

2 Reference

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

3 Definition

nitrate and nitrite contents of caseins and caseinates: 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

Dispersion of the casein or caseinate in warm water, precipitation of the fat and proteins, and filtration.

Reduction of the nitrate to nitrite 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 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 set of sodium nitrite calibration 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 hydrochloric acid solution, $c(HCl) \approx 0,1$ mol/l, 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 **Hydrochloric acid** solution, $c(HCl) \approx 2$ mol/l.

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

5.4 **Hydrochloric acid** solution, $c(HCl) \approx 0,1$ mol/l.

Dilute 50 ml of the hydrochloric acid (5.3) to 1 000 ml with water.

5.5 Solutions for precipitation of proteins and fat.

5.5.1 **Zinc sulfate** solution.

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

5.5.2 **Potassium hexacyanoferrate(II)** solution.

Dissolve 17,2 g of potassium hexacyanoferrate(II) trihydrate ($K_4[Fe(CN)_6] \cdot 3H_2O$) in water and dilute to 100 ml.

5.6 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.

5.7 Solutions for colour development.

5.7.1 Solution I.

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

5.7.2 Solution II.

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.

5.7.3 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. Filter if necessary.

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

5.8 Sodium nitrite, standard solution corresponding to 0,001 g of NO_2^- per litre.

5.8.1 Stock solution.

Dry a few grams of sodium nitrite ($NaNO_2$) at 110 to 120 °C to constant mass, i.e. until the difference between two successive weighings does not exceed 1 mg. Dissolve 0,150 g of the sodium nitrite in water in a 1 000 ml one-mark volumetric flask. Dilute to the mark with water and mix.

Prepare this solution on the day of use.

5.8.2 Preparation of standard solution.

Transfer, by means of pipettes, 10 ml of the stock solution (5.8.1) and 20 ml of the buffer solution (5.10) to a 1 000 ml one-mark volumetric flask. Dilute to the mark with water and mix.

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

5.9 Potassium nitrate, standard solution corresponding to 0,004 5 g of NO_3^- per litre.

5.9.1 Stock solution.

Dry a few grams of potassium nitrate (KNO_3) at 110 to 120 °C to constant mass, i.e. until the difference between two successive weighings does not exceed 1 mg. Dissolve 1,468 g of the potassium nitrate in water in a 1 000 ml one-mark volumetric flask. Dilute to the mark with water and mix.

5.9.2 Preparation of standard solution.

Transfer, by means of pipettes, 5 ml of the stock solution (5.9.1) and 20 ml of the buffer solution (5.10) to a 1 000 ml one-mark volumetric flask. Dilute to the mark with water and mix.

Prepare this solution on the day of use.

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

5.10 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 solution] with 600 ml of water. After mixing, add 135 ml of ammonia solution [ϱ_{20} 0,91 g/ml; about 25 % (m/m) NH_3 solution]. Dilute to 1 000 ml with water and mix.

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

NOTE — If ammonia solution 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).

6 Apparatus

NOTE ON THE PREPARATION OF GLASSWARE

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

Usual laboratory equipment and in particular

6.1 Analytical balance.

6.2 Sample container, provided with an airtight lid.

6.3 Magnetic stirrer.

6.4 Conical flasks, of capacities 250 and 500 ml.

6.5 One-mark 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 Measuring cylinders, of capacities 5; 10; 25; 100; 250; 500; and 1 000 ml.

6.8 Glass funnels, of diameter 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.

6.12 Test sieve, of woven wire cloth, diameter 200 mm, nominal size of openings 500 µm, with receiver, complying with the requirements of ISO 565.

6.13 Grinding device, for grinding the laboratory sample if necessary. This device should be such that no undue heat will be developed and no loss of moisture occurs. (A hammer mill shall not be used.)

7 Sampling

See ISO 707.

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 250 ml conical flask (6.4).

8.1.2 Add sufficient of the 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 (i.e. until reaction with silver nitrate is negative).

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 shall 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 potassium nitrate solution (5.9), 20 ml of the buffer solution (5.10) and 20 ml of the EDTA solution (5.6), 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 (5.9) into the reservoir on top of the column. Immediately add 5 ml of the buffer solution (5.10) to the contents of the reservoir. Collect the eluate in a 100 ml one-mark 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, dilute to the mark with water and mix well.

8.2.4 Pipette 10 ml of the eluate into a 100 ml one-mark volumetric flask. Add water to obtain a volume of about 60 ml. Proceed as specified in 8.10.2, 8.10.3 and 8.10.4.

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 graph (8.7), 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 %, regenerate the column.

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 (5.6) and 2 ml of the hydrochloric acid solution (5.4) 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 successively with water, the hydrochloric acid solution (5.4) and water.

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

8.4.1 Thoroughly mix the laboratory sample (clause 7), if necessary after transferring all of it to an airtight container (6.2) of suitable capacity, by repeatedly shaking and inverting the container.

8.4.2 Transfer 50 g of the laboratory sample to the test sieve (6.12). If it does not pass completely through the sieve, use the grinding device (6.13) to achieve this condition. Immediately transfer all the sieved sample to the container (6.2) and mix thoroughly in the closed container. During these operations, take precautions to avoid any change in the water content of the product.

8.4.3 After the test sample has been prepared, proceed with the determination (starting at 8.5) as soon as possible.

If the 50 g portion directly passes, or nearly completely passes, the sieve, use the prepared test sample (see 8.4.1) for the determination.

8.5 Test portion

Weigh, to the nearest 0,01 g, approximately 10 g of the sample in the case of caseins or 2 g of the test sample in the case of caseinates, and transfer quantitatively into a 500 ml conical flask (6.4).

8.6 Blank test

Carry out a blank test in parallel with the operations specified in 8.8, 8.9 and 8.10.1 to 8.10.3 inclusive, by the same procedure, using the same quantities of all the reagents, but omitting the test portion.

8.7 Calibration graph

8.7.1 Pipette 0; 2; 4; 6; 8; 12; and 20 ml of the sodium nitrite standard solution (5.8) into separate 100 ml one-mark volumetric flasks. Add water to each volumetric flask to obtain volumes of about 60 ml.

8.7.2 Carry out the procedures specified in 8.10.2 and 8.10.3.

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

8.7.4 Plot the absorbances obtained in 8.7.3 against the nitrite concentrations, in micrograms per millilitre, calculated from the amounts of standard sodium nitrite solution added (see 8.7.1).

8.8 Extraction and deproteination

8.8.1 Add progressively 136 ml of warm (50 to 55 °C) water and 10 ml of the buffer solution (5.10) to the test portion and dissolve the casein and caseinate by stirring, using the magnetic stirrer (6.3).

8.8.2 Add, in the following order, 12 ml of the zinc sulfate solution (5.5.1), 12 ml of the potassium hexacyanoferrate(II) solution (5.5.2) and 30 ml of the buffer solution (5.10), swirling thoroughly after each addition.

8.8.3 Leave for at least 15 min but no longer than 1 h. Then filter through a filter paper (6.9), collecting the filtrate in a 250 ml conical flask.

NOTE — It is essential to obtain a clear filtrate and it may thus be necessary to leave the solution for more than 15 min to satisfy this requirement.

8.9 Reduction of nitrate to nitrite

8.9.1 Pipette 20 ml of the filtrate (8.8.3) into the reservoir on top of the reduction column. Add 5 ml of the buffer solution (5.10) to the contents of the reservoir and mix by stirring with a small glass rod. Collect the eluate in a 100 ml one-mark volumetric flask (6.5). The flow rate shall not exceed 6 ml/min.

8.9.2 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.

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

8.10 Determination

8.10.1 Pipette equal volumes (for example 25 ml) of the filtrate (8.8.3) and of the eluate (8.9.3) into separate 100 ml one-mark volumetric flasks. Add water to each to obtain a volume of about 60 ml. Then treat the contents of each flask as specified in 8.10.2, 8.10.3 and 8.10.4.

8.10.2 Add 6 ml of solution I (5.7.1) and then 5 ml of solution II (5.7.2). Mix carefully and leave the solution for 5 min at room temperature, protected from direct sunlight.

8.10.3 Add 2 ml of solution III (5.7.3). Mix carefully and leave the solution for 5 min at room temperature, protected from direct sunlight. Dilute to the mark with water and mix.

8.10.4 Measure within 15 min the absorbance of the solution against that of the blank test (8.6) at a wavelength of 538 nm.

9 Expression of results

9.1 Nitrite content

9.1.1 Method of calculation and formula

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

$$\frac{20\,000 \times c_1}{m \times V}$$

where

c_1 is the concentration, in micrograms of nitrite ion per millilitre, read from the calibration graph, corresponding to the measured absorbances (8.10.4) of the solution obtained using the filtrate (8.8.3);

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

V is the volume, in millilitres, of the aliquot portion taken (8.10.1) from the filtrate (8.8.3).

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

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

$$1{,}35 \left[\frac{100\,000 \times c_2}{m \times V} - w(NO_2^-) \right]$$

where

c_2 is the concentration, in micrograms of nitrite ion per millilitre, read from the calculation graph, corresponding to the measured absorbance (8.10.4) of the solution obtained using the eluate (8.9.3);

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

V is the volume, in millilitres, of the aliquot portion taken (8.10.1) from the eluate (8.9.3);

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

Report the result to the nearest 1 mg/kg.

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

$$1{,}35 \left[\frac{100\,000 \times c_2}{m \times V} - w(NO_2^-) \right] \frac{100}{r}$$

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

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.

10 Test report

The test report shall show the method used and the results obtained. It shall also mention any operating details not specified in this International Standard, or regarded as optional, together with details of any incidents likely to have influenced the results.

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

Dimensions in millimetres

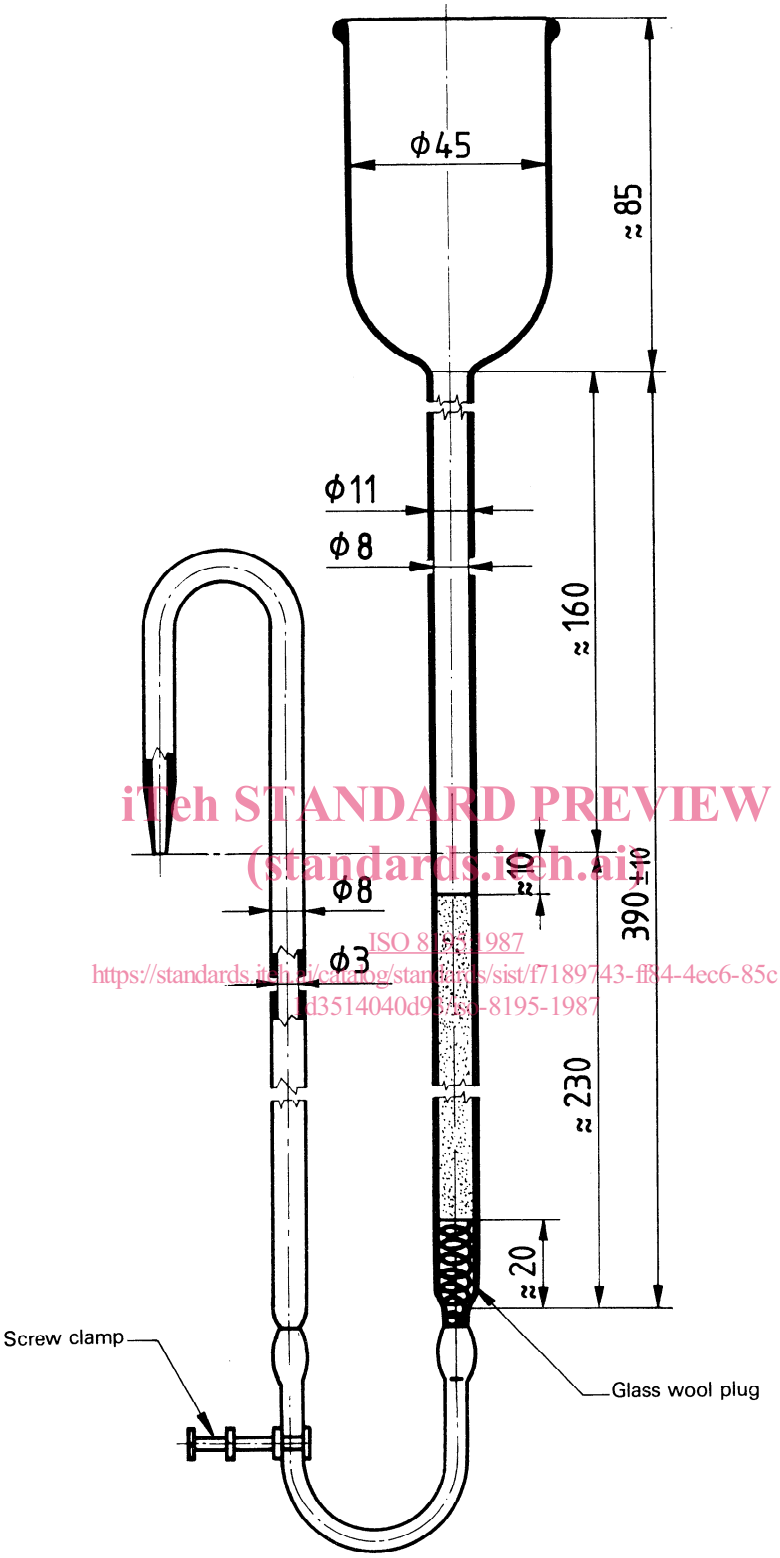

Figure — Apparatus for nitrate reduction

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Descriptors : agricultural products, dairy products, caseins, chemical analysis, determination of content, nitrates, nitrites, spectrochemical analysis, test equipment.

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