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Dried milk — Determination of nitrate content — Method by cadmium reduction and spectrometry (Screening method)

Lait sec — Détermination de la teneur en nitrates — Méthode par réduction au cadmium et spectrométrie (Méthode rapide)

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 8151 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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Dried milk — Determination of nitrate content — Method by cadmium reduction and spectrometry (Screening method)

1 Scope and field of application

This International Standard specifies a screening method by cadmium reduction and spectrometry for the determination of the nitrate content of dried milk.

2 Reference

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

3 Definition

nitrate content of dried milk: The content of substances determined by the procedure specified in this International Standard and expressed as milligrams of nitrate ion (NO_3^-) per kilogram.

NOTE — Any nitrite present in the dried milk will be determined as nitrate.

4 Principle

Dissolution of the dried milk in water, precipitation of the fat and proteins, and filtration.

Reduction of the nitrate to nitrite in a portion of the filtrate by means of zinc powder and cadmium ion.

Development of a red colour in a portion of the supernatant liquid by addition of sulfanilamide and *N*-1-naphthyl ethylenediamine dihydrochloride, and spectrometric measurement at a wavelength of 538 nm.

5 Reagents

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

5.1 Solutions for precipitation of proteins and fat.

5.1.1 Zinc sulfate solution.

Dissolve 267,5 g of zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) in water and dilute to 500 ml.

5.1.2 Potassium hexacyanoferrate(II) solution.

Dissolve 86 g of potassium hexacyanoferrate(II) trihydrate ($K_4[Fe(CN)_6] \cdot 3H_2O$) in water and dilute to 500 ml.

5.2 Cadmium acetate solution.

Dissolve 0,5 g of cadmium acetate dihydrate $[(CH_3COO)_2Cd \cdot 2H_2O]$ in water, add 1 ml of acetic acid (CH_3COOH) and dilute to 100 ml.

5.3 Zinc, suspension.

Just before use, introduce 10 ml of water and 2 g of zinc powder into a small beaker. Maintain the zinc in suspension by magnetic stirring.

5.4 Solutions for colour development.

5.4.1 Solution I.

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

5.4.2 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.

5.4.3 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.

5.5 Potassium nitrate, standard solution corresponding to 0,03 g of NO_3^- per litre.

5.5.1 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 489,2 mg of the potassium nitrate in water in a 1 000 ml one-mark volumetric flask. Dilute to the mark with water and mix.

5.5.2 Preparation of standard solution.

Transfer, by means of a pipette, 10 ml of stock solution (5.5.1) to a 100 ml one-mark volumetric flask. Dilute to the mark with water and mix.

Prepare this standard solution on the day of use.

1 ml of this standard solution contains 30,0 µg of NO_3^-.

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

Add, in a 1 000 ml one-mark volumetric flask, 17 ml of concentrated sulfuric acid [ϱ_{20} 1,84 g/ml; about 98 % (m/m) H_2SO_4 solution] to approximately 600 ml of water. Cool to room temperature and mix. Add 100 ml of concentrated ammonia solution [ϱ_{20} 0,91 g/ml; about 25 % (m/m) NH_3 solution]. Dilute to the mark with water and mix.

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

5.7 Silicone antifoam.[1)]

6 Apparatus

NOTE ON PREPARATION OF GLASSWARE

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

Usual laboratory equipment and in particular

6.1 Spectrometer, suitable for measuring absorbance at a wavelength of 538 nm, with cells of optical path length 1 to 4 cm.

6.2 Centrifuge, capable of producing a relative radial acceleration of at least 200g.

6.3 Centrifuge tubes, ground-glass stoppered, of minimum capacity 25 ml, for use with the centrifuge (6.2).

6.4 Magnetic stirrer.

6.5 Filter papers, medium grade, of diameter about 15 cm, free from nitrate and nitrite.

6.6 Conical flasks, of capacity 500 ml.

6.7 One-mark volumetric flasks, of capacity 100 and 1 000 ml, complying with the requirements of ISO 1042, class B.

6.8 Pipettes, to deliver 5; 10; 15; and 20 ml, complying with the requirements of ISO 648, class A, or ISO 835/1.

7 Sampling

See ISO 707.

8 Procedure

8.1 Preparation of the test sample

Transfer the dried milk into a container of capacity about twice the volume of the powder, provided with an airtight lid. Close the container immediately. Mix the dried milk thoroughly by repeatedly shaking and inverting the container.

8.2 Test portion

Weigh, to the nearest 0,01 g, approximately 10 g of the test sample and transfer quantitatively into a 500 ml conical flask (6.6).

8.3 Blank test

Carry out a blank test in parallel with the operations specified in 8.5 to 8.7.3 inclusive, by the same procedure, using the same quantities of all the reagents, but omitting the test portion.

8.4 Calibration graph

8.4.1 Place 10 g of dried skimmed milk, almost free from nitrate and nitrite, in each of five conical 500 ml flasks (6.6). Add gradually 136; 131; 126; 121; and 116 ml, respectively, of warm (50 to 55 °C) water and dissolve the dried skimmed milk by stirring with glass rods or by shaking the flasks. Add to each flask 0,1 ml of the silicone antifoam (5.7).

Pipette respectively 0; 5; 10; 15; and 20 ml of the potassium nitrate standard solution (5.5) into the five conical flasks.

8.4.2 In each case, proceed as specified in 8.5.2 to 8.7.3 inclusive. Measure the absorbances of the treated filtrates from the flasks to which standard solutions were added against that of the zero member (the treated filtrate from the flask to which no standard solution was added).

8.4.3 Plot the measured absorbances against the masses, in micrograms, of nitrate added (0; 150; 300; 450; and 600 µg, respectively).

8.5 Extraction and deproteination

8.5.1 Add gradually 136 ml of warm (50 to 55 °C) water to the test portion and dissolve the powder by stirring with a glass rod or by shaking the flask. Add 0,1 ml of the silicone antifoam (5.7).

[1)] A suitable product, available commercially, is Fluka 85390. This information is given for the convenience of the user of this International Standard and does not constitute an endorsement of this product by ISO.

8.5.2 Add, in order, 12 ml of the zinc sulfate solution (5.1.1) and 12 ml of the potassium hexacyanoferrate(II) solution (5.1.2), swirling thoroughly after each addition. Add 40 ml of the buffer solution (5.6) and swirl thoroughly. Filter through a fluted filter paper (6.5).

NOTE — It is essential that the filtrate is clear and it may thus be necessary to leave the solution for some time to satisfy this requirement.

8.6 Reduction of nitrate to nitrite

8.6.1 Pipette 20 ml of the filtrate (8.5.2) into a centrifuge tube (6.3). Add 0,5 ml of the cadmium acetate solution (5.2). Mix.

8.6.2 Add approximately 0,5 ml of the zinc suspension (5.3). Immediately after this addition swirl the tube and regularly turn it upside down for 5 min, but avoid mixing with air. If necessary, centrifuge the tube at a relative radial acceleration of at least 200*g* for 5 min.

8.7 Determination

8.7.1 Using a syringe or a pipette (6.8), carefully transfer 10 ml of the supernatant liquid (8.6.2) into a 100 ml volumetric flask (6.7).

8.7.2 Add 5 ml of solution I (5.4.1) and then 5 ml of solution II (5.4.2). Mix carefully and leave the solution for 5 min at room temperature, protected from direct sunlight.

8.7.3 Add 2 ml of solution III (5.4.3). 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.

8.7.4 Measure within 15 min the absorbance of the solution against that of the blank test (8.3) at a wavelength of 538 nm.

If the absorbance is higher than 1,5, repeat the determination after diluting the filtrate obtained in 8.5.

9 Expression of results

9.1 Method of calculation and formula

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

$$\frac{m_1}{m_0}$$

where

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

m_1 is the mass, in micrograms, of nitrate, corresponding to the absorbance measured in 8.7.4, read from the calibration graph (8.4).

Report the result to the nearest 1 mg/kg.

9.2 Repeatability

The difference between two results obtained within a short time interval by the same analyst should not exceed 6 mg/kg if the nitrate content is less than 30 mg/kg, and should not exceed 20 % 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 test report shall include all the information necessary for the complete identification of the sample.

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