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IDF 189-2

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Milk and milk products — Determination of nitrate and nitrite contents —

Part 2: Method using segmented flow analysis (Routine method)

Lait et produits laitiers — Détermination des teneurs en nitrates et en nitrites —

Partie 2: Méthode d'analyse par flux continus segmentés (Méthode de routine)

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 14673-2|IDF 189-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

This second edition cancels and replaces the first (ISO 14673-2|IDF 189-2:2001), of which it constitutes a minor revision.

ISO 14673|IDF 189 consists of the following parts, under the general title *Milk and milk products — Determination of nitrate and nitrite contents*:

— *Part 1: Method using cadmium reduction and spectrometry*

— *Part 2: Method using segmented flow analysis (Routine method)*

— *Part 3: Method using cadmium reduction and flow injection analysis with in-line dialysis (Routine method)*

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO and AOAC International in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the National Committees casting a vote.

ISO 14673-2 | IDF 189-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Minerals and minor compounds*, of the Standing Committee on *Minor components characterization of physical properties*, under the aegis of its project leader, Mr G. Bråthen (NO).

This second edition, together with ISO 14673-1 | IDF 189-1 and ISO 14673-3 | IDF 189-3, cancels and replaces IDF 84A:1984, IDF 95A:1982, IDF 96A:1987, IDF 97A:1985 and IDF 120:1984, which have been technically revised.

Milk and milk products — Determination of nitrate and nitrite contents —

Part 2:
Method using segmented flow analysis (Routine method)

WARNING — The use of this International Standard may involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This part of ISO 14673|IDF 189 specifies a routine method for the determination of the nitrate and nitrite contents of milk and milk products by segmented flow analysis. The method is applicable to milk, cheese, and liquid and dried milk products and infant foods.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 648, *Laboratory glassware — One-mark pipettes*

ISO 835-1, *Laboratory glassware — Graduated pipettes — Part 1: General requirements*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
nitrate content
mass fraction of nitrate determined by the procedure specified in this part of ISO 14673|IDF 189

NOTE The nitrate content is expressed as the mass in milligrams of nitrate ions (NO_3^-) per kilogram of product.

3.2
nitrite content
mass fraction of nitrite determined by the procedure specified in this part of ISO 14673|IDF 189

NOTE The nitrite content is expressed as the mass in milligrams of nitrite ions (NO_2^-) per kilogram of product.

4 Principle

4.1 Nitrate determination

A test portion is suspended in water. Part of the suspension is transferred to the analyser for dialysis. The nitrate ions are reduced to nitrite. The nitrite content is determined by a spectrometric method.

Standard nitrate solutions are determined by the same procedure. The nitrate content is calculated by comparing the reading obtained from the test portion with the readings from the standard solutions.

NOTE Any nitrite present is determined as nitrate. The amount of nitrite is generally small compared to the amount of nitrate. Infant food based on soy-proteins can be an exception to the rule. A correction for the nitrite present can be applied after determination of the nitrite content.

4.2 Nitrite determination

A test portion is suspended in a solution of ammonium and sodium chloride. Part of the suspension is transferred to the analyser for dialysis. The nitrite content is determined by a spectrometric method.

Standard nitrite solutions are determined by the same procedure. The nitrite content is calculated by comparing the reading obtained from the test portion with that from the standard solution.

5 Reagents

Use only reagents of recognized analytical grade unless otherwise specified.

5.1 Water, distilled or deionized, or water of equivalent purity, free from nitrate and nitrite ions.

To avoid the possible inclusion of small gas bubbles in the copperized cadmium column (6.9), freshly boil the distilled or deionized water and cool to room temperature. Use the thus-prepared water for the preparation of the column (9.1), to check the reducing capacity of the column (9.2), and for regeneration of the column.

5.2 Cadmium granules, of diameter 0,3 mm to 0,8 mm.

Prepare cadmium granules, if not available commercially, as follows.

Place a suitable number of zinc rods in a beaker. Cover the rods with cadmium sulfate solution (5.3). Scrape the cadmium sponge from the rods from time to time over a period of 24 h. Remove the zinc rods and decant the liquid until only sufficient remains to cover the cadmium sponge. Wash the sponge two or three times with water. Transfer the cadmium sponge to a laboratory blender together with 400 ml of the dilute hydrochloric acid (5.6) 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, while stirring occasionally to remove bubbles. Decant most of the liquid and immediately copperize the granules as described in 9.1.2.

WARNING — Because of its toxicity, the used cadmium should be delivered as chemical waste to the relevant authorities.

5.3 Cadmium sulfate solution, $c(CdSO_4 \cdot 8H_2O) = 40$ g/l.

Dissolve 40 g of cadmium sulfate in water in a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.4 Copper(II) sulfate solution, $c(CuSO_4 \cdot 5H_2O) = 20$ g/l.

Dissolve 2 g of copper(II) sulfate in water in a 100 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.5 Hydrochloric acid (HCl), ($\rho_{20} = 1{,}19$ g/ml).

5.6 Dilute hydrochloric acid, c(HCl) ≈ 1 mol/l.

Carefully add 80 ml of hydrochloric acid (5.5) to about 700 ml water in a 1 000 ml volumetric flask (6.4) while regularly swirling the contents. Cool the contents to room temperature. Dilute to the mark with water and mix carefully.

5.7 Disodium ethylenediaminetetraacetate dihydrate (EDTA) solution ($Na_2C_{10}H_{14}N_2O_8{\cdot}2H_2O$).

Dissolve 33,5 g of EDTA in about 900 ml water in a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.8 Poly(oxyethylene lauryl ether) solution, 30 % mass fraction.

5.9 Ammonium and sodium chloride solution I.

Dissolve 40 g of ammonium chloride (NH_4Cl) and 200 g of sodium chloride (NaCl) in about 950 ml water in a 1 000 ml volumetric flask (6.4). Use concentrated ammonia to adjust the pH to 8,5. Dilute to the mark with water. Add 2 ml of poly(oxyethylene lauryl ether) solution (5.8) and mix.

5.10 Ammonium and sodium chloride solution II.

Dissolve 10 g of ammonium chloride (NH_4Cl) and 50 g of sodium chloride (NaCl) in 950 ml water in a 1 000 ml volumetric flask (6.4). Add 20 ml EDTA solution (5.7) and mix. Use concentrated ammonia to adjust the pH to 8,5. Dilute to the mark with water. Add 2 ml of poly(oxyethylene lauryl ether) solution (5.8) and mix.

5.11 Ammonium and sodium chloride solution III.

Dissolve 10 g of ammonium chloride (NH_4Cl) and 50 g of sodium chloride (NaCl) in 950 ml water in a 1 000 ml volumetric flask (6.4). Use concentrated ammonia to adjust the pH to 8,5. Dilute the contents to the 1 000 ml mark with water. Add 2 ml of poly(oxyethylene lauryl ether) solution (5.8) and mix.

5.12 Phosphoric acid solution (H_3PO_4), 85 %, (ρ_{20} = 1,71 g/ml).

5.13 Colour reagent.

Add 100 ml of phosphoric acid solution (5.12) to 800 ml water in a 1 000 ml volumetric flask (6.4). Add in the following order: 10 g sulfanilamide ($NH_2C_6H_4SO_2NH_2$) and 0,5 g *N*-1-naphthylethylene diamine dihydrochloride ($C_{10}H_7NHCH_2CH_2NH_2{\cdot}2HCl$) and mix. Dilute to the mark with water. Add 0,5 ml of poly(oxyethylene lauryl ether) solution (5.8) and mix again.

If stored in a refrigerator, the colour reagent solution may be kept for up to 1 month.

5.14 Sodium nitrate stock solution ($NaNO_3$), $c(NO_3^-)$ = 0,400 g/l.

Dry an amount of sodium nitrate in the oven (6.18) set at 110 °C to 120 °C for 2 h. Dissolve 0,548 4 g of the dry sodium nitrate in water in a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.15 Sodium nitrate working solution, $c(NO_3^-)$ = 40 µg/ml.

Pipette (6.5) 10 ml of the sodium nitrate stock solution (5.14) into a 100 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.16 Sodium nitrate calibration solutions.

Prepare a series of sodium nitrate calibration solutions with an increasing concentration of nitrate by pipetting into six 200 ml volumetric flasks respectively, 1 ml, 2 ml, 3 ml, 6 ml, 9 ml and 12 ml of the sodium nitrate working solution (5.15). Dilute each solution to the mark with water and mix. The nitrate (NO_3^-) contents of the obtained sodium nitrate calibration solutions are 0,2 µg/ml, 0,4 µg/ml, 0,6 µg/ml, 1,2 µg/ml, 1,8 µg/ml and 2,4 µg/ml respectively.

5.17 Sodium nitrite stock solutions ($NaNO_2$).

Dry an amount of sodium nitrite in the oven (6.18) set at 110 °C to 120 °C for 2 h.

5.17.1 Sodium nitrite stock solutions I, $c(NO_2^-) = 0{,}297$ mg/ml.

Dissolve 0,445 g of the dry sodium nitrite (5.17) in water in a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix. Stock solution I may be stored for up to 1 day.

5.17.2 Sodium nitrite stock solutions II, $c(NO_2^-) = 1{,}001$ mg/ml.

Dissolve 1,502 g of sodium nitrite (5.17) in water in a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix. If stored in a refrigerator, stock solution II may be kept for up to 1 month.

5.18 Sodium nitrite working solutions.

5.18.1 Sodium nitrite working solution I, $c(NO_2^-) = 0{,}89$ µg/ml.

Transfer with a pipette (6.5) 3 ml of the sodium nitrite stock solution I (5.17.1) to a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.18.2 Sodium nitrite working solution II, $c(NO_2^-) = 0{,}100\ 1$ µg/ml.

Pipette (6.5) 10 ml of the sodium nitrite stock solution II (5.17.2) into a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix. Pipette 10 ml of this solution into another 1 000 ml volumetric flask (6.4). Dilute to the mark with ammonium and sodium chloride solution III (5.11) and mix.

5.19 Sodium nitrite calibration solutions.

Prepare a series of sodium nitrite calibration solutions with an increasing concentration of nitrite by pipetting into five 200 ml volumetric flasks (6.4) respectively, 10 ml, 20 ml, 40 ml, 100 ml and 200 ml of sodium nitrite working solution II (5.18.2). Except for the 200 ml solution, dilute each solution to the 200 ml mark with ammonium and sodium chloride solution III (5.11) and mix. The sodium nitrite calibration solutions have a nitrite (NO_2^-) content of 0,005 µg/ml, 0,010 µg/ml, 0,020 µg/ml, 0,050 µg/ml and 0,100 µg/ml, respectively.

5.20 Alkaline detergent.

Use one of the solutions mentioned below.

5.20.1 Extran solution, 5 % volume fraction.

Pipette (6.5) 50 ml of extran solution into a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.20.2 Sodium hydroxide solution, $c(NaOH) = 0{,}1$ mol/l.

Dissolve 4 g of sodium hydroxide (NaOH) in water in a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.21 Regenerating fluid.

Add 50 ml of EDTA solution (5.7) and 2,0 ml of dilute hydrochloric acid (5.6) to 1 litre of water (5.1) and mix.

5.22 Reference test sample.

Use as a reference test sample, samples from a milk powder with a known nitrate content.

5.23 pH indicator paper.

6 Apparatus

Clean all glassware thoroughly and rinse with distilled water to ensure that it is free from nitrate and nitrite ions.

Usual laboratory equipment and, in particular, the following.

6.1 Analytical balance, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.

6.2 Sample container, provided with an airtight lid.

6.3 Conical flasks, of capacity 250 ml and 500 ml.

6.4 Volumetric flasks, of nominal capacity 100 ml, 500 ml and 1 000 ml, complying with the requirements of ISO 1042, class B.

6.5 Pipettes, capable of delivering 1 ml, 2 ml, 3 ml, 6 ml, 9 ml, 10 ml, 12 ml, 20 ml, 40 ml, 50 ml and 100 ml, complying with the requirements of ISO 648, class A, or ISO 835-1. Where appropriate, burettes may be used instead of pipettes.

6.6 Graduated cylinders, of capacities 100 ml and 150 ml.

6.7 Glass beakers, of capacities 100 ml and 150 ml.

6.8 Glass funnels, with a stem of internal diameter about 2 mm.

6.9 Suitable tube, U-shaped, of length about 30 cm and internal diameter 2 mm.

6.10 Analyser, capable of determining the nitrate and nitrite contents according to the described procedure.

6.11 Suspension apparatus, appropriate to suspend the test samples.

6.12 Grinding device, appropriate for grinding the laboratory sample, if necessary.

To avoid loss of moisture the device should not produce undue heat. A hammer mill shall not be used.

6.13 Laboratory mixer or **homogenizer**, with glass containers of capacity 250 ml or 400 ml, suitable for suspending test portions of cheese and whey cheese.

6.14 Glass wool.

6.15 Spatula.

6.16 Magnetic stirrer.

6.17 Water baths, capable of being maintained at 35 °C to 40 °C and of boiling water.

6.18 Oven, capable of being maintained at 110 °C to 120 °C.

7 Sampling

Sampling is not part of the method specified in this part of ISO 14673|IDF 189. A recommended sampling method is given in ISO 707.

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

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