INTERNATIONAL STANDARD

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IDF 189-3

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

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

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

Partie 3: Méthode par réduction au cadmium et d'analyse par injection avec dialyse en ligne (Méthode de routine)

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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. Each 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. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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 part of ISO 14673 | IDF 189 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 14673-3 | IDF 189-3 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 first edition of ISO 14673-3 | IDF 189-3, together with ISO 14673-1 | IDF 189-1 and ISO 14673-2 | IDF 189-2, cancels and replaces ISO 4099:1984, ISO 6736:1982, ISO 6739:1988, ISO 6740:1985 and ISO 8195:1987, which have been technically revised.

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 National Committees casting a vote.

International Standard ISO 14673-3 | IDF 189-3 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).

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

Part 3:
Method using cadmium reduction and flow injection analysis with in-line dialysis (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 cadmium reduction and flow injection analysis (FIA). The method is applicable to hard, semi-hard and soft cheeses of various ages, and processed cheese. The detection limits of the method are 0,5 mg of nitrate ions per kilogram and 1,0 mg of nitrite ions per kilogram, respectively.

The method is also applicable to whey powder, milk powder and milk-based infant food.

NOTE 1 The method closely resembles the FIA method described in reference [2] for the determination of nitrate and nitrite in milk and fluid dairy products. Adaptations were made to allow for the analysis of cheese and to obtain sufficient sensitivity for the determination of low levels of nitrite in cheese and milk-based infant foods.

NOTE 2 For determination of nitrite and nitrate following cadmium reduction, use is made of the same colour reaction as described in ISO 14673-1|IDF 189-1.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 14673|IDF 189. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 14673|IDF 189 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

ISO 14673-1|IDF 189-1, *Milk and milk products — Determination of nitrate and nitrite contents — Part 1: Method using cadmium reduction and spectrometry*

3 Terms and definitions

For the purposes of this part of ISO 14673|IDF 189, 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 A test portion is suspended in warm extraction buffer. Fat is separated by centrifuging and rapid cooling. Analyses are made of small portions of the de-fatted solution by flow injection analysis (FIA). In-line dialysis is used to remove protein and remaining fat. The nitrate ions are reduced to nitrite ions by cadmium. The nitrite ions are reacted with sulfanilamide and *N*-1-naphthyl ethylenediamine dihydrochloride to give a red-coloured azo dye. The colour is measured in a flow cell at maximum absorption of the dye at 540 nm with reference to the absorption measured at 620 nm.

4.2 The nitrite and nitrate contents of the test sample are calculated with reference to the measured absorbances for a series of standard solutions of nitrite and nitrate, respectively. If the nitrite content exceeds 0,5 mg/kg, or exceeds 10 % of the nitrate content, correction of the nitrate content is made by subtracting the nitrite content from the obtained nitrate results.

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.

5.2 **Cadmium reduction column**, for example Aquatec-Tecator [1].

5.3 **Extraction buffer solution** or **carrier solution (C2)**.

Dissolve 26,6 g of ammonium chloride (NH_4Cl) in 800 ml water in a 1 000 ml conical flask. By adding concentrated ammonia, adjust the pH to 8,5. Dilute to 1 000 ml with water and mix.

5.4 **Hydrochloric acid** (HCl), (ρ_{20} = 1,19 g/ml).

5.5 **Reagent solution (R1)**.

Dissolve 5,0 g sulfanilamide ($NH_2C_6H_4SO_2NH_2$) in a mixture of 300 ml water and 26 ml of hydrochloric acid (5.4) in a 500 ml volumetric flask (6.3). Dilute to the mark with water and mix.

5.6 **Reagent solution (R2)**.

Dissolve 0,5 g of *N*-1-naphthyl ethylenediamine dihydrochloride ($C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$) in water in a 500 ml volumetric flask (6.3). Dilute to the mark with water and mix.

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

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1) Aquatec-Tecator is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 14673 | IDF 189 and does not constitute an endorsement by ISO of this product.

5.7 Regeneration solution, c(HCl) = 0,1 mol/l.

Dilute 80 ml of hydrochloric acid (5.4) with water to 1 litre and mix.

5.8 Sodium nitrate standard solution, $c(NO_3^-)$ = 1 000 mg/l.

Before use, dry sodium nitrate ($NaNO_3$) to constant mass at 110 °C to 120 °C. Dissolve 137,1 mg of the dry $NaNO_3$ in water in a 100 ml volumetric flask (6.3). Dilute to the mark with water and mix.

5.9 Sodium nitrate calibration standard solutions.

Prepare the sodium nitrate calibration standard solution on the day of use. Pipette amounts of 25 µg/l, 50 µg/l, 100 µg/l, 150 µg/l and 250 µg/l of sodium nitrate standard solution (5.8) into separate 50 ml volumetric flasks (6.3). Dilute to the mark with extraction buffer solution (5.3) to obtain sodium nitrate calibration standards solutions corresponding to 0,50 mg/l, 1,00 mg/l, 2,00 mg/l, 3,00 mg/l and 5,00 mg/l nitrate ions respectively.

5.10 Sodium nitrite standard solution, $c(NO_2^-)$ = 1 000 mg/l.

Before use, dry the sodium nitrite ($NaNO_2$) to constant mass at 110 °C to 120 °C. Prepare the sodium nitrite standard solution on the day of use. Dissolve 150,0 mg of the dry $NaNO_2$ in water in a 100 ml volumetric flask (6.3). Dilute to the mark with water and mix.

5.11 Sodium nitrite standard solution, $c(NO_2^-)$ = 50,0 mg/l.

Pipette 5,00 ml of the sodium nitrite standard solution (5.10) in a 100 ml volumetric flask. Dilute to the mark with water and mix.

5.12 Sodium nitrite calibration standard solution.

Shortly before use, pipette amounts of 25 µl, 50 µl, 100 µl, 200 µl and 400 µl of the sodium nitrite standard solution (5.11) into separate 50 ml volumetric flasks (6.3). Dilute to the mark with extraction buffer solution (5.3) to obtain sodium nitrite calibration standard solutions corresponding to 0,025 mg/l, 0,050 mg/l, 0,100 mg/l, 0,200 mg/l and 0,400 mg/l nitrite ions respectively. Mix well.

5.13 Sodium nitrite reference solution, $c(NO_2^-)$ = 1,48 mg/l.

Pipette 1 480 µl of the sodium nitrite standard solution (5.11) in a 50 ml volumetric flask (6.3). Dilute to the mark with extraction buffer solution (5.3) and mix. Prepare the nitrite reference solution shortly before use.

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 Volumetric flasks, of nominal capacity 50 ml, 100 ml and 500 ml respectively, complying with the requirements of ISO 1042, class B.

6.4 Pipettes, capable of delivering 25 µl, 50 µl, 100 µl, 150 µl, 200 µl, 250 µl, 400 µl and 1 480 µl respectively (semi-automatic pipettes).

6.5 Grinding device, appropriate for grinding the test sample, if necessary. To avoid loss of moisture, the device should not produce undue heat. A hammer mill shall not be used.