INTERNATIONAL STANDARD

ISO 8968-3

IDF 20-3

First edition
2004-09-01

Milk — Determination of nitrogen content —

Part 3: Block-digestion method (Semi-micro rapid routine method)

Lait — Détermination de la teneur en azote —

Partie 3: Méthode de minéralisation en bloc (Méthode de routine semi-micro rapide)

Reference numbers
ISO 8968-3:2004(E)
IDF 20-3:2004(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org
Web www.fil-idf.org

Published in Switzerland

Contents

Page

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 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 8968-3|IDF 20-3 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.

ISO 8968|IDF 20 consists of the following parts, under the general title *Milk — Determination of nitrogen content*:

— *Part 1: Kjeldahl method*

— *Part 2: Block-digestion method (Macro method)*

— *Part 3: Block-digestion method (Semi-micro rapid routine method)*

— *Part 4: Determination of non-protein-nitrogen content*

— *Part 5: Determination of protein-nitrogen content*

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 IDF National Committees casting a vote.

ISO 8968-3 | IDF 20-3 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, *Nitrogen compounds,* of the Standing Committee on *Main components of milk,* under the aegis of its project leader Mrs S. Berman (IL).

This edition of ISO 8968-3 | IDF 20-3 cancels and replaces IDF 20-3:1993, which has been technically revised.

ISO 8968 | IDF 20 consists of the following parts, under the general title *Milk — Determination of nitrogen content*:

— *Part 1: Kjeldahl method*

— *Part 2: Block-digestion method (Macro method)*

— *Part 3: Block-digestion method (Semi-micro rapid routine method)*

— *Part 4: Determination of non-protein-nitrogen content*

— *Part 5: Determination of protein-nitrogen content*

Milk — Determination of nitrogen content —

Part 3:
Block-digestion method (Semi-micro rapid routine method)

WARNING — The use of this part of ISO 8968|IDF 20 may involve the use of hazardous materials, operations and equipment. This standard does not purport to address all the safety risks associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of local regulatory limitations prior to use.

1 Scope

This part of ISO 8968|IDF 20 specifies a method for the determination of the nitrogen content of liquid, whole or skimmed milk.

It concerns a semi-micro rapid routine method following the block-digestion principle.

NOTE The method is a more rapid method than that described in ISO 8968-1|IDF 20-1 and ISO 8968-2|IDF 20-2 since the digestion time is reduced by taking a lower mass of test portion and using hydrogen peroxide together with sulfuric acid and a catalyst in the digestion.

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 385:—[1)], *Laboratory glassware — Burettes*

3 Terms and definitions

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

3.1
nitrogen content
mass fraction of nitrogen determined by the procedure specified in this part of ISO 8968|IDF 20.

NOTE The nitrogen content is expressed as a percentage by mass.

1) To be published. (Revision of ISO 385-1:1984, ISO 385-2:1984 and ISO 385-3:1984)

4 Principle

A test portion is digested by using a block-digestion apparatus with a mixture of concentrated sulfuric acid, hydrogen peroxide and potassium sulfate, together with a catalyst to convert the organic nitrogen present to ammonium sulfate. Excess sodium hydroxide is added to the cooled digest to liberate ammonia.

The liberated ammonia is distilled using a manual, semi-automatic or fully automatic steam distillation unit. In the case of manual or semi-automatic steam distillation, the ammonia is distilled into excess boric acid solution then titrated with hydrochloric acid solution. Where a fully automatic distillation unit is employed, titration of the ammonia is carried out automatically with endpoint detection using a photometric or pH system. The nitrogen content is calculated from the amount of ammonia produced.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water.

5.1 Kjeldahl catalyst tablets

These tablets may be purchased commercially. Tablets comprising 3,5 g of potassium sulfate, 0,105 g of copper(II) sulfate pentahydrate and 0,105 g of titanium dioxide per tablet are suitable.

Other types of tablet may be used provided that

a) they contain a quantity of potassium sulfate such that 7 g of potassium sulfate can be dispensed using an integer number of whole tablets, and

b) they contain no salts of toxic metals such as selenium or mercury.

5.2 Sulfuric acid (H_2SO_4), with mass fraction of at least 95 % to 98 %, nitrogen free [$\rho_{20}(H_2SO_4)$ = 1,84 g/ml approximately].

5.3 Hydrogen peroxide solution (H_2O_2), approximately 30 g of H_2O_2 per 100 ml.

5.4 Antifoaming agent

A silicone preparation is recommended, for example with a mass fraction of 30 % aqueous emulsion.

5.5 Sodium hydroxide solution (NaOH), nitrogen free, containing approximately 40 g of NaOH per 100 ml.

5.6 Boric acid solutions

5.6.1 Boric acid solution, $c(H_3BO_3)$ = 40,0 g/l.

Dissolve 40,0 g of boric acid in 1 litre of hot water in a 1 000 ml one-mark volumetric flask. Allow the flask and its contents to cool to 20 °C. Add 3 ml of indicator solution (5.7.1). Adjust to the mark with water and mix well.

Store the solution, which will be light orange in colour, in a borosilicate glass bottle. Protect the solution from light and sources of ammonia fumes during storage.

5.6.2 Boric acid solution, $c(H_3BO_3)$ = 10,0 g/l, to be used in the photometric endpoint titration.

Dissolve 10,0 g of boric acid in 1 litre of hot water in a 1 000 ml one-mark volumetric flask. Allow the flask and its contents to cool to 20 °C. Add 7 ml of methyl red solution (5.7.2) and 10 ml of bromocresol green solution (5.7.2) and mix.

Dilute to the 1 000 ml mark with water and mix well. Neutralize the boric acid solution with 0,1 mol/l sodium hydroxide until its colour changes to green.

NOTE The addition of 3 ml of 0,1 mol/l NaOH into 1 litre of 1 % boric acid usually gives good adjustments.

5.7 Indicator solutions

5.7.1 Indicator solution to be used in the pH endpoint titration

Dissolve 0,1 g of methyl red in 95 % (volume fraction) ethanol. Dilute to 50 ml with the ethanol. Dissolve 0,5 g of bromocresol green in a small quantity of 95 % (volume fraction) ethanol. Dilute to 250 ml with the ethanol.

Mix amounts of one part of methyl red solution with five parts of bromocresol green solution, or combine and mix both solutions.

5.7.2 Indicator solution to be used in the boric acid solution (5.6.2) for the photometric endpoint titration

a) Dissolve 0,1 g of bromocresol green in 100 ml of 95 % (volume fraction) ethanol.

b) Dissolve 0,1 g of methyl red in 100 ml of 95 % (volume fraction) ethanol.

5.8 Hydrochloric acid standard volumetric solution, c(HCl) = (0,1 ± 0,0005) mol/l.

If the titration (9.2.3) is carried out manually, the use of a more diluted hydrochloric acid standard volumetric solution, c(HCl) = (0,05 ± 0,0005) mol/l, is recommended.

5.9 Ammonium sulfate [$(NH_4)_2SO_4$], minimum assay 99,9 % (mass fraction) on dried material.

Immediately before use, dry the ammonium sulfate at 102 °C ± 2 °C for not less than 2 h. Cool to room temperature in a desiccator.

5.10 Tryptophan ($C_{11}H_{12}N_2O_2$) or lysine hydrochloride ($C_6H_{15}ClN_2O_2$), minimum assay 99 % (mass fraction).

Do not dry the reagents in an oven before use.

5.11 Sucrose, with a nitrogen content of not more than 0,002 % (mass fraction).

Do not dry the sucrose in an oven before use.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Water bath, capable of being maintained at between 38 °C and 40 °C.

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

6.3 Digestion block, aluminium alloy block or equivalent block, fitted with an adjustable temperature control and device for measuring block temperature.

6.4 Digestion tubes, of capacity 250 ml, suitable for use with the digestion block (6.3).

6.5 Exhaust manifold, suitable for use with the digestion tubes (6.4).