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Milk — Determination of nitrogen content —

Part 1: Kjeldahl method

Lait — Détermination de la teneur en azote
Partie 1: Méthode Kjeldahl

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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.

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 part of ISO 8968 | IDF 20 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 8968-1 | IDF 20-1 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 the non-protein-nitrogen content*

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

Annex A of this part of ISO 8968 | IDF 20 is for information only.

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 8968-1 | IDF 20-1 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*, under the aegis of its project leader, Mr D.M. Barbano (US).

Milk — Determination of nitrogen content —

Part 1:
Kjeldahl 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 healthy 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 milk, whole or skimmed, by the Kjeldahl principle.

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this part of ISO 8968 | IDF 20. 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 8968 | IDF 20 are encouraged to investigate the possibility of applying the most recent edition of the normative document 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 385-1, *Laboratory glassware — Burettes — Part 1: General requirements*

3 Term and definition

For the purposes of this part of ISO 8968 | IDF 20, the following term and definition 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.

4 Principle

A test portion is digested with a mixture of concentrated sulfuric acid and potassium sulfate, using copper(II) sulfate as a catalyst to thereby convert organic nitrogen present to ammonium sulfate. The function of the potassium sulfate is to elevate the boiling point of the sulfuric acid and to provide a stronger oxidizing mixture for digestion. Excess sodium hydroxide is added to the cooled digest to liberate ammonia.The liberated ammonia is distilled into excess boric acid solution then titrated with hydrochloric acid. 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 or water of equivalent purity.

5.1 Potassium sulfate (K_2SO_4), nitrogen free.

5.2 Copper(II) sulfate solution, $c(CuSO_4)$ = 5,0 g per 100 ml.

Dissolve 5,0 g of copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in water in a 100 ml one-mark volumetric flask. Dilute to the mark with water and mix.

5.3 Sulfuric acid (H_2SO_4), with a mass fraction of at least 95 % to 98 %, nitrogen free (ρ_{20} = 1,84 g/ml approximately).

5.4 Sodium hydroxide solution (NaOH), nitrogen free, containing 50 g of sodium hydroxide per 100 g of solution.

5.5 Indicator solution

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 95 % (volume fraction) ethanol. Dilute to 250 ml with the ethanol. Mix amounts of one part of the methyl red solution with five parts of the bromocresol green solution or combine and mix all of both solutions.

5.6 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. Dilute to the mark with water, add 3 ml of the indicator solution (5.5) and mix. 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.

If using the electronic pH endpoint titration, the addition of the indicator solution to the boric acid solution may be omitted. On the other hand, the change in colour may also be used as a check of proper titration procedures.

5.7 Hydrochloric acid standard volumetric solution, $c(HCl)$ = (0,1 ± 0,000 5) mol/l.

It is recommended that this material be purchased prestandardized by the manufacturer to meet or exceed the above specification.

NOTE Often systematic errors (which can be avoided) introduced by an analyst diluting a concentrated stock acid and then determining the molarity of the acid, can reduce the reproducibility of the method. The analyst should not use a solution for titration that has a higher concentration than 0,1 mol/l, because this will reduce the total titration volume per sample and the uncertainty in readability of the burette will become a larger percentage of the value. This will have a negative impact on the repeatability and reproducibility of the method. The same issues and additional sources of error arise when another acid (e.g. sulfuric acid) is substituted for hydrochloric acid. Thus, these substitutions are not recommended.

5.8 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.

5.9 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 these reagents in an oven before use.

5.10 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 38 °C ± 2 °C

6.2 Kjeldahl flasks, of capacity 500 ml or 800 ml.

6.3 Analytical balance, capable of weighing to the nearest 0,1 mg.

6.4 Boiling aids, e.g. glowed pumice, zinc dust, hard pieces of porcelain or high-purity amphoteric alundum (i.e. carbarundum) granules, plain, mesh size 10.

Do not reuse the aids.

NOTE Glass beads of approximately 5 mm diameter are sometimes used, but they might not promote as efficient boiling as the alundum granules and more foaming problems can be encountered during digestion with glass beads.

6.5 Burette or **automatic pipette,** capable of delivering 1,0 ml portions of the copper sulfate solution (5.2).

6.6 Graduated measuring cylinders, of capacity 50 ml, 100 ml and 500 ml.

6.7 Digestion apparatus, to hold the Kjeldahl flasks (6.2) in an inclined position (at approximately 45°), with electric heaters or gas burners that do not heat the flasks above the level of their contents, and with a fume extraction system.

The heater source should be adjustable to control the maximum heater setting to be used during digestion. Preheat the heat source at the heater setting for evaluation. In the case of a gas heater, the preheated period shall be 10 min, and for an electric heater it shall be 30 min. For each of the heaters, determine the heater setting that brings 250 ml of water including 5 to 10 boiling aids with an initial temperature of 25 °C to its boiling point in 5 min to 6 min. This is the maximum heater setting to be used during digestion.

6.8 Distillation apparatus, made of borosilicate glass or other suitable material to which can be fitted a Kjeldahl flask (6.2) consisting of an efficient splash-head connected to an efficient condenser with straight inner tube and an outlet tube attached to its lower end.

The connecting tubing and stopper(s) shall be close fitting and preferably made of neoprene.

6.9 Conical flasks, of capacity 500 ml, graduated at every 200 ml.

6.10 Burette, of capacity 50 ml, graduated at least at every 0,01 ml, complying with the requirements of ISO 385-1, class A.

Alternatively, an automatic burette may be used if it fulfils the same requirements.

6.11 Automatic titrator provided with a pH-meter

The pH-meter should be correctly calibrated in the range of pH 4 to 7 following normal laboratory pH-calibration procedures.

7 Sampling

Sampling is not part of the method specified in this part of ISO 8968 | IDF 20. 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.