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**Milk — Determination of nitrogen
content —**
Part 2:
Block-digestion method (Macro method)

Lait — Détermination de la teneur en azote —
Partie 2: Méthode de minéralisation en bloc (Méthode macro)
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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 8968-2 | IDF 20-2 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.

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*

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 8968-2|IDF 20-2 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, *Nitrogen compounds*, under the aegis of its project leader, Mr D.M. Barbano (US).

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Milk — Determination of nitrogen content —

Part 2:

Block digestion method (Macro 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 block-digestion 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 substances 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 by using a block-digestion apparatus 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 environment. Excess sodium hydroxide is added to the cooled digest to liberate ammonia. The liberated ammonia is steam distilled, using either a manual or semi-automatic steam distillation unit, into an excess of 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 between 95 % to 98 %, nitrogen free ($\rho_{20} = 1,84$ g/ml approximately).

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

A 40 % sodium hydroxide solution may be used instead of a 50 %, if plugging of the flow system in an automatic distillation unit is a problem.

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, $c(HCl) = (0,1 \pm 0,000 5)$ 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 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 $102 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ 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 the 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 $38\text{ °C} \pm 2\text{ °C}$.
- 6.2 Analytical balance**, capable of weighing to the nearest 0,1 mg.
- 6.3 Digestion block**, aluminium alloy block or equivalent block, fitted with an adjustable temperature control and device for measuring the temperature of the block.
- 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).
- 6.6 Centrifugal scrubber apparatus or filter pump or aspirator**, constructed of acid-resistant material, for use with mains water supply.
- 6.7 Burette or automatic pipette**, capable for delivering 1,0 ml portions of the copper sulfate solution (5.2).
- 6.8 Graduated measuring cylinders**, of capacity 25 ml, 50 ml and 100 ml.
- 6.9 Distillation unit**, capable of steam distilling, manual or semi-automatic, suited to accept the 250 ml digestion tubes (6.4) and the 500 ml conical flasks (6.10).
- 6.10 Conical flasks**, of capacity 500 ml, graduated at every 200 ml.
- 6.11 Burette**, of capacity 50 ml, graduated at least at every 0,01 ml, complying with the requirements of ISO 385-1, class A.

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

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6.12 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.

8 Preparation of test sample

Warm the test sample in the water bath (6.1) set at 38 °C . Gently mix the test sample thoroughly by repeatedly inverting the sample bottle without causing frothing or churning. Cool the sample to room temperature immediately prior to weighing the test portion (9.1).

NOTE For advice on sample size to apply this method to dairy products other than milk, see annex A of ISO 8968-1 | IDF 20-1:2001.