INTERNATIONAL STANDARD

ISO 8968-5

IDF 20-5

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Milk — Determination of nitrogen content —

Part 5: Determination of protein-nitrogen content

Lait — Détermination de la teneur en azote —

Partie 5: Détermination de la teneur en azote protéique

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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.ch
Web www.iso.ch

International Dairy Federation
41 Square Vergote • 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

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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 8968-5 | IDF 20-5 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 8968-5 | IDF 20-5 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 5:
Determination of protein-nitrogen content

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 direct determination of the protein-nitrogen content of liquid milk, whole or skimmed.

An alternative indirect method using calculations is also described.

2 Normative references

The following normative documents contain 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 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 8968-1|IDF 20-1:2001, *Milk — Determination of nitrogen content — Part 1: Kjeldahl method*

ISO 8968-2|IDF 20-2:2001, *Milk — Determination of nitrogen content — Part 2: Block-digestion method (Macro method)*

ISO 8968-4|IDF 20-4:2001, *Milk — Determination of nitrogen content — Part 4: Determination of the non-protein-nitrogen content*

3 Term and definition

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

3.1
protein-nitrogen content
mass fraction of substances determined by the procedure specified in this part of ISO 8968|IDF 20, directly or indirectly

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

4 Principle

Protein is precipitated from a test portion by addition of trichloroacetic acid solution such that the final concentration of trichloroacetic acid in the mixture is approximately 12 %.The protein precipitate is separated by filtration. (The filtrate contains the non-protein-nitrogen contents.) The nitrogen content of the filtrate is determined by the procedure described either in part 1 or part 2 of ISO 8968 | IDF 20.

5 Reagents

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

The reagents are as specified for the determination of total nitrogen described in part 1 or part 2 of ISO 8968 | IDF 20, together with the following. A laboratory may decide which method to use.

5.1 Trichloroacetic acid solution (CCl_3COOH)

Dissolve 15,0 g of trichloroacetic acid in water in a 100 ml one-mark volumetric flask. Dilute to the mark with water. Do not use concentrations of trichloroacetic acid and volumes of solutions other than those specified.

The performance of the method with respect to mean value and between-laboratory performance characteristics will differ if other than the specified concentrations of trichloroacetic acid and volumes of solutions are used.

5.2 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.

6 Apparatus

Usual laboratory apparatus and, in particular, the following, together with the apparatus specified in part 1 or part 2 of ISO 8968 | IDF 20.

6.1 Water bath, capable of being maintained at 38 °C ± 2 °C.

6.2 Pipette, of capacities 5 ml.

6.3 Filter funnel, made of glass, of diameter 75 mm.

6.4 Filter paper, nitrogen free, of diameter 15 cm (e.g. Whatman No. 1[1)]) or equivalent.

6.5 Automatic pipette or **piston pump**, capable of delivering 10 ml.

7 Sampling

Sampling is not part of the method specified in this International Standard. 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.

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1) Whatman is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 8968 | IDF 20 and does not constitute an endorsement by ISO of this product.

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).

9 Procedure

9.1 Test portion

Pipette 5,0 ml ± 0,1 ml of the prepared test sample (clause 8) either into a dry and clean Kjeldahl flask or digestion tube, preweighed to the nearest 0,1 mg. Weigh the test sample to the nearest 0,1 mg. Immediately add 5 ml ± 0,1 ml of water to the flask or tube, rinsing any test sample on its neck into the flask or tube.

NOTE 1 The use of either a Kjeldahl flask or digestion tube depends on the laboratory's choice of method.

NOTE 2 For advice on test portion size to apply this method to dairy products other than milk, see annex A of ISO 8968-1 | IDF 20-1:2001.

9.2 Direct determination

9.2.1 Precipitation and filtration

Add 40 ml ± 0,5 ml of trichloroacetic acid solution (5.1) to the Kjeldahl flask or digestion tube containing the test portion (9.1) and swirl to mix the contents. Let the flask or tube stand for approximately 5 min to allow the precipitate to settle. Pour the contents of the flask or tube through a filter paper (6.4) placed in a filter funnel (6.3). Collect the filtrate in a clean conical flask. Some of the precipitate will remain in the Kjeldahl flask or digestion tube and some will be collected on the filter paper. It is not necessary to remove all of the precipitate from the flask or tube.

Immediately after pouring the mixture and so as not to allow any precipitate to dry on the neck of the flask or tube, add by means of an automatic pipette (6.5), 10 ml of the trichloroacetic acid solution (5.1). Use the solution to rinse any precipitate from the neck of the flask or tube down into it. Swirl to mix the contents. Pour the thus-obtained contents of the flask or tube through the same filter paper. Add the filtrate to that collected previously in the conical flask. Again, immediately rinse the neck of the flask or tube with a further 10 ml of trichloroacetic acid solution and swirl to mix the contents. Pour the contents of the flask or tube for the third time through the same filter paper, adding the filtrate to that collected previously in the conical flask.

The obtained filtrate shall be clear and free of particulate matter. At this point, the filtrate is no longer needed and may be discarded in an appropriate manner.

If duplicated tests of the same test sample are to be done, then two separate precipitation and filtration procedures shall be carried out for each test sample.

9.2.2 Preparation of the filtrate

Wearing gloves, carefully remove the filter paper from the filter funnel and fold the paper to enclose the precipitate. If any precipitate remains on either the inner or outer lip of the Kjeldahl flask or digestion tube, wipe with the folded filter paper so that any precipitate adheres to the paper and then drop the filter paper also into the Kjeldahl flask or digestion tube.

9.2.3 Digestion and distillation

Add the appropriate amount of boiling aids, potassium sulfate, coper catalyst solution and sulfuric acid either to the Kjeldahl flask or digestion tube and continue with the digestion and distillation by the procedure described in either part 1 or part 2 of ISO 8968 | IDF 20.

A laboratory may choose to use either part 1 or part 2 of ISO 8968 | IDF 20.

9.2.4 Blank test

Carry out a blank test, taking a filter paper (6.4) washed with trichloroacetic acid solution (5.1) instead of the test portion as described in 9.2.1 and proceeding to 9.2.3. Always titrate the blanks with the same reagent and apparatus as used for the test portions.

Keep a record of the blank values. If the blank values change, identify the cause.

9.3 Indirect determination

Alternatively, a classical indirect determination of the protein-nitrogen content of a test sample can be calculated. This may be done by subtracting the non-protein-nitrogen content determined by the method given in ISO 8968-4 | IDF 20-4 from the total nitrogen content of the same test sample determined by the method given either part 1 or part 2 of ISO 8968 | IDF 20. The obtained result for the protein-nitrogen content is multiplied by 6,38, if expressed as the true protein content.

10 Calculation and expression of results

10.1 Calculation of protein-nitrogen content

10.1.1 Calculate the protein-nitrogen content of the test sample, w_{pn}, by the following equation:

$$w_{pn} = \frac{1{,}4007\,(V_s - V_b)\,M_r}{m}$$

where

w_{pn} is the protein-nitrogen content of the sample, expressed as a percentage by mass;

V_s is the numerical value of the volume, in millilitres, of the hydrochloric acid (5.2) used in the determination, expressed to the nearest 0,05 ml;

V_b is the numerical value of the volume, in millilitres, of the hydrochloric acid (5.2) used in the blank test, expressed to the nearest 0,05 ml;

M_r is the numerical value of the exact molarity of the hydrochloric acid (5.2), expressed to four decimal places;

m is the numerical value of the mass, in grams, of the test portion (9.1), expressed to the nearest 0,1 mg;

10.1.2 Express the obtained results to four decimal places, if needed for further calculations. In the case of end results, express those obtained for the nitrogen content to three decimal places and for the protein content to two decimal places. The obtained results should not be rounded further until the final use of the test value is made.

NOTE This is particularly true when the values are to be used for further calculations. One example is when the individual test values obtained from the analysis of many sample materials are used to calculate method performance statistics for within- and between-laboratory variation. Another example is when the values are used as a reference for instrument calibration (e.g. infrared milk analyser) where the values from many samples will be used in a simple or multiple regression calculation. In such cases the obtained results should not be rounded before they are used for further calculations.

10.2 Calculation of true protein content

10.2.1 Calculate the true protein content of the test sample, w_p, using the following equation:

$$w_p = w_{pn} \times 6{,}38$$

where

w_p is the true protein content, expressed as a percentage by mass;

w_{pn} is the protein-nitrogen content of the sample, expressed as a percentage by mass to four decimal places (10.1);

6,38 is the generally accepted multiplication factor to express the nitrogen content as true protein content.

10.2.2 Express the obtained results for the true protein content to three decimal places, if needed for further calculations. In the case of end results (see 10.1), express these to two decimal places.

11 Precision

11.1 Interlaboratory test

The values for the repeatability and reproducibility were derived from the result of an interlaboratory test carried out in accordance with ISO 5725[2]. Details of the interlaboratory test of the method are summarized in references [5], [6]. The values derived from this test may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than 0,003 8 % for protein-nitrogen content (0,024 % for true protein content).

11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than 0,009 2 % for protein-nitrogen content (0,059 % for true protein content).

12 Test report

The test report shall specify:

— all information required for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, with reference to whether part 1 or part 2 of ISO 8968 | IDF 20 was used;

— all operating details not specified in this part of ISO 8968 | IDF 20, or regarded as optional, together with details of any incident which may have influenced the result(s);

— the test result(s) obtained;

— if the repeatability has been checked the final quoted result obtained;

— if the recovery has been checked the final quoted result obtained.

2) ISO 5725:1986 (now withdrawn), was used to obtain the precision data.