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IDF 20-4

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Milk — Determination of nitrogen content —

Part 4: Determination of non-protein-nitrogen content

Lait — Détermination de la teneur en azote —

Partie 4: Détermination de la teneur en azote non protéique

Reference numbers
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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 8968-4 | IDF 20-4 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 8968-4 | IDF 20-4 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 4:
Determination of non-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 determination of the non-protein nitrogen content of liquid milk, whole or skimmed.

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)*

3 Term and definition

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

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

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

4 Principle

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

NOTE Where the total nitrogen content of the milk sample has previously been determined, the true protein-nitrogen content can be calculated as the difference between the total nitrogen content and the non-protein-nitrogen content.

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 by the method described either in part 1 or part 2 of ISO 8968 | IDF 20, together with the following. A laboratory may decide whether to use the method described in part 1 or part 2 of ISO 8968 | IDF 20.

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) = (0,01 ± 0,000 1) 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 reduce the reproducibility of the method. The analyst should not use solution for titration that has a higher concentration than 0,01 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.

6 Apparatus

Usual laboratory apparatus and, in particular, the following, together with the apparatus as specified in either part 1 or part 2 of ISO 8968 | IDF 20, depending on the method used.

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

6.2 Conical flasks, of capacity 125 ml.

6.3 Pipettes, of capacities 10 ml and 20 ml.

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

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

6.6 Beaker, of capacity 50 ml.

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.

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).

9 Procedure

9.1 Test portion

Pipette 10,0 ml ± 0,1 ml of the prepared test sample (clause 8) into a preweighed conical flask (6.2), weighed to the nearest 0,1 mg, then reweigh it to the nearest 0,1 mg.

NOTE 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 Determination

9.2.1 Precipitation and filtration

Add 40 ml ± 0,5 ml of trichloroacetic acid solution (5.1) to the conical flask containing the test portion (9.1). Weigh the flask and its contents to the nearest 0,1 mg. Swirl to mix. Let the flask stand for approximately 5 min to allow the precipitate to settle.

Filter the contents of the conical flask through a filter paper (6.5) placed in a filter funnel (6.4). Collect the entire filtrate in a clean, dry conical flask (6.2). The filtrate shall be clear and free of particulate matter. If it is not, repeat the process of precipitation and filtration with a new test portion.

If duplicate 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

Swirl the filtrate in the conical flask (9.2.1) to ensure that it is mixed. Pipette 20 ml of the filtrate into a 50 ml beaker (6.6) and weigh. Pour the filtrate from the beaker into a Kjeldahl flask or digestion tube containing boiling aid, potassium sulfate and copper sulfate(II) solution as specified either in part 1 or part 2 of ISO 8968 | IDF 20. Immediately reweigh the empty beaker to the nearest 0,1 mg.

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

9.2.3 Digestion and distillation

Add the appropriate amount of sulfuric acid to the Kjeldahl flask or digestion tube and continue with the digestion and distillation procedure specified in either part 1 or part 2 of ISO 8968 | IDF 20.

9.2.4 Titration

Titrate the liberated ammonia by using the procedure specified either in part 1 or part 2 of ISO 8968 | IDF 20 but replacing the 0,1 mol/l hydrochloric acid by the 0,01 mol/l hydrochloric acid (5.2).

9.3 Blank test

Digest, distil and titrate blanks comprising about 0,1 g of sucrose and 16 ml ± 0,5 ml of trichloroacetic acid solution (5.1).

Always titrate blanks with the same reagent and apparatus as used for the test portions. Carry out a blank test following the procedures described in either part 1 or part 2 of ISO 8968 | IDF 20. Replace the test portion with16 ml ± 0,5 ml of trichloroacetic acid solution (5.1) and 0,1 g of sucrose.

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

10 Calculation and expression of results

10.1 Calculate the non-protein-nitrogen content of the test sample, w_{np}, by the following equation:

$$w_{np} = \frac{1{,}4007\,(V_s - V_b)\,M_r}{m_f\ m_m/(m_t - 0{,}065\ m_m)}$$

where

w_{np} is the non-protein-nitrogen (NPN) 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 (5.1) 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 (5.1) 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 (5.1), expressed to four decimal places;

m_f is the numerical value of the mass, in grams, of 20 ml of filtrate (9.2.2), expressed to the nearest 0,1 mg;

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

m_t is the numerical value of the mass, in grams, of the test portion with the addition of 40 ml of trichloroacetic acid solution (9.2.1) expressed to the nearest 0,1 mg.

0,065 is the multiplication factor, based on the assumption that milk contains a mass fraction of about 3,5 % fat and 3,0 % true protein (thus 0,035 + 0,030 = 0,065).

NOTE The multiplication factor 0,065 in the denominator may need to be adjusted if liquid dairy products of different composition are analysed (e.g. concentrated or fractionated skim or whole milk products.)

10.2 Express the obtained results to four decimal places, if needed for further calculations. In the case of end results, express those obtained for the non-protein-nitrogen content to three decimal places, and for the non-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.

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 reference [5]. 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 0,002 5 % for NPN 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 0,005 2 % for NPN 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.