DRAFT INTERNATIONAL STANDARD

ISO/DIS 8968-4

ISO/TC **34**/SC **5**

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Milk and milk products — Determination of nitrogen content —

Part 4:
Determination of protein and non protein nitrogen content and true protein content calculation (Reference method)

Lait et produits laitiers — Détermination de la teneur en azote —

Partie 4: Détermination de la teneur en azote protéique et non protéique et calcul des protéines vraies (Méthode de référence)

[Revision of first edition (ISO 8968-4:2001)]

ICS: 67.100.10

ISO/CEN PARALLEL PROCESSING

This draft has been developed within the International Organization for Standardization (ISO), and processed under the **ISO lead** mode of collaboration as defined in the Vienna Agreement.

This draft is hereby submitted to the ISO member bodies and to the CEN member bodies for a parallel five month enquiry.

Should this draft be accepted, a final draft, established on the basis of comments received, will be submitted to a parallel two-month approval vote in ISO and formal vote in CEN.

To expedite distribution, this document is circulated as received from the committee secretariat. ISO Central Secretariat work of editing and text composition will be undertaken at publication stage.

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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 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 8968-4 was prepared by Technical Committee ISO/TC 34, *Food and food products*, Subcommittee SC 5, *Milk and milk products and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.*

The ISO 8968-4 | IDF 20-4:2013 cancels and replaces the editions ISO 8968-4 | IDF 20-4:2001 and ISO 8968-5 | IDF 20-5:2001.

ISO 8968 | IDF 20 consists of the following parts, under the general title *Milk — Determination of protein nitrogen content and true protein calculation (Reference method)*:

— Part 1: Kjeldahl principle – to be published

— Part 3: Block digestion method (Semi micro rapid routine method):2004;

— Part 4:Determination of protein and non-protein nitrogen content and true protein content calculation (Reference method) – to be published

Foreword

IDF (the International Dairy Federation) is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented at the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

The main task of Standing Committees is to prepare International Standards. Draft International Standards adopted by the Standing Committees are circulated to the National Committees for endorsement prior to publication as an International Standard. Publication as an International Standard requires approval by at least 50% of IDF National Committees casting a vote.

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All work was carried out by Joint ISO-IDF Project Group (C13) of the Standing Committee on Analytical methods for Composition (SCAMC) under the aegis of its project leaders, D. Barbano (US) and P. Trossat (FR).

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Milk — Determination of protein nitrogen content and true protein calculation (Reference method)

WARNING — The use of this part of ISO 8968 | IDF 20 may involve the use of hazardous materials, operations, and equipment. This part does not purport to address all the safety risks associated with its use. It is the responsibility of the user of this part of ISO 8968 | IDF 20 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 and indirect determination of the protein-nitrogen content of liquid, whole or skimmed milk.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard.

ISO 8968-1 | IDF 20-1, *Milk - Determination of nitrogen content. Part 1: Kjeldahl principle*

3 Terms and definitions

For the purposes of this part of ISO 8968 | IDF 20 the following definitions apply.

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

3.2
Protein nitrogen content
Mass fraction of substances determined by the procedure specified in this part of ISO 8968 | IDF 20, directly or alternatively indirectly.

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

4 Principle

4.1 Indirect protein nitrogen

Precipitation of protein from a test portion by addition of trichloroacetic acid solution such that the final concentration of trichloroacetic acid in the mixture is approximately 12 %. Removal of the precipitated milk protein by filtration, the remaining filtrate containing the non-protein nitrogen components. Determination of the nitrogen content of the filtrate by the procedure described in part 1 of ISO 8968 | IDF 20.

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.

4.2 Direct protein nitrogen

Precipitation of protein from a test portion by addition of trichloroacetic acid solution such that the final concentration of trichloroacetic acid in the mixture is approximately 12 %. Separation of the protein precipitate by filtration. (The precipitate contains the protein nitrogen content of the sample.) Determination of the nitrogen content of the precipitate by the procedure described in part 1 of ISO 8968 | IDF 20.

5 Reagents

5.1 General

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

The reagents specified for the determination of total nitrogen by the method described in part 1 of ISO 8968 | IDF 20, with the exception of 0,10 mol/l hydrochloric acid standard volumetric solution, together with the following are required.

NOTE Depending on the laboratory's choice whether the determination of the nitrogen in the protein precipitate should be carried out by the method described in part 1 of ISO 8968 | IDF 20, the reagents described in that method are required too.

5.2 Trichloroacetic acid (CCl_3COOH) solution

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.

NOTE The performance of the method with respect to mean value and between laboratory performance characteristics will be different, if using other than specified concentrations of trichloroacetic acid and volumes of solutions.

5.3 Hydrochloric acid standard volumetric solution

For the direct approach the 0,1 mol/l hydrochloric acid is as described in part 1 of ISO 8968 | IDF 20.

For the indirect protein nitrogen approach the following hydrochloric acid is required c(HCl) = (0,01 ± 0,0001) mol/l, in addition to the 0,1 mol/l hydrochloric acid required in part 1 of ISO 8968 | IDF 20.

NOTE: It is recommended to purchase this material pre-standardized by the manufacturer that meets, or exceeds, these specifications. Many times the systematic errors (that can be avoided) introduced by an analyst diluting a concentrated stock acid and then determining the molarity of the acid, cause poor reproducibility performance of the method in this part. The analyst should not use a solution for titration that has a higher concentration than 0,1 mol/l, because this may reduce the total titration volume per sample and the uncertainty in readability of the burette will become a larger percentage of the value. This may have a negative impact on the method repeatability and reproducibility performance. If sulfuric acid is substituted for hydrochloric acid the solution should have a concentration of 0,05 ± 0.0003 mol/l.

6 Apparatus

6.1 General

Usual laboratory apparatus and, those specified for the determination of total nitrogen described in part 1 of ISO 8968 | IDF 20 and, in particular, the following.

NOTE Depending on the laboratory's choice as mentioned in the note to 5.1, the apparatus described in the chosen method is required.

6.2 Water bath, capable of maintaining a temperature of between 38 °C and 40 °C.

6.3 Conical flasks, of capacity 125 ml (indirect approach only)

6.4 Pipettes, of capacities 5 ml, 10 ml, and 20 ml.

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

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

6.7 Automatic pipette, piston pump, capable of delivering 10 ml.

6.8 Beakers, of capacity 50 ml. (indirect approach only).

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707 | IDF 50.

It is important that the laboratory receives a sample which is representative and has not been damaged or changed during transport or storage.

8 Preparation of test sample

Warm the test sample to between 38 °C to 40 °C in the water bath (6.2). Cool the sample to room temperature while gently mixing the test sample immediately prior to weighing the test portion (9.1).

9 Procedure - Direct protein nitrogen approach

9.1 Test portion

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

NOTE The use of either a Kjeldahl flask or digestion tube is depending the laboratory's choice as mentioned in the note to 5.1.

9.2 Determination

9.2.1 Precipitation and filtration

Add 40 ml ± 0,5 ml of trichloroacetic acid solution (5.2) 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 (6.6) placed in a filter funnel (6.5). 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 (6.7), 10 ml of the trichloroacetic acid solution (5.2). Use the solution to rinse any precipitate from the neck of the flask or tube down into the bottom. 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.

[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.