INTERNATIONAL STANDARD

ISO 8968-4

IDF 20-4

Second edition
2016-04-01

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)

iTeh STANDARD PREVIEW
(standards.iteh.ai)

Lait et produits laitiers — Détermination de la teneur en azote — Partie 4: Détermination de la teneur en azote protéique et non protéique et calcul de la teneur en protéines vraies (Méthode de référence)

ISO 8968-4:2016
https://standards.iteh.ai/catalog/standards/sist/ad8f8310-0260-4b67-a560-9921549a4e11/iso-8968-4-2016

Reference numbers
ISO 8968-4:2016(E)
IDF 20-4:2016(E)

© ISO and IDF 2016

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 8968-4:2016
https://standards.iteh.ai/catalog/standards/sist/ad8f8310-0260-4b67-a560-9931549a4e11/iso-8968-4-2016

COPYRIGHT PROTECTED DOCUMENT

© ISO and IDF 2016, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

International Dairy Federation
Silver Building • Bd Auguste Reyers 70/B • B-1030 Brussels

Tel. + 32 2 325 67 40
Fax + 32 2 325 67 41
info@fil-idf.org
www.fil-idf.org

© ISO and IDF 2016 – All rights reserved

Contents

Page

Forewords ..... **v**

1 Scope **1**

2 Normative references **1**

3 Terms and definitions **1**

4 Principle **1**
4.1 Indirect protein nitrogen 1
4.2 Direct protein nitrogen 2

5 Reagents **2**
5.1 General 2
5.2 Trichloroacetic acid (CCl_3COOH) solution 2
5.3 Hydrochloric acid standard volumetric solution 2

6 Apparatus **2**

7 Sampling **3**

8 Preparation of test sample **3**

9 Procedure — Direct protein nitrogen approach **3**
9.1 Test portion 3
9.2 Determination 3
9.2.1 Precipitation and filtration 3
9.2.2 Preparation of the precipitate 4
9.2.3 Digestion and distillation 4
9.2.4 Titration 4
9.3 Blank test 4
9.4 Calculation and expression of results 4
9.4.1 Calculation of protein nitrogen content 4
9.4.2 Expression of results 5
9.4.3 Calculation of true protein content 5
9.4.4 Expression of the true protein content results 5

10 Procedure — Indirect protein nitrogen approach **6**
10.1 Nitrogen determination 6
10.2 Non-protein nitrogen determination 6
10.2.1 Test portion 6
10.2.2 Precipitation and filtration 6
10.2.3 Preparation of the filtrate 6
10.2.4 Digestion and distillation 6
10.2.5 Titration 6
10.3 Blank test 6
10.4 Calculation and expression of results 7
10.4.1 Calculation of non-protein nitrogen content 7
10.4.2 Calculation of protein nitrogen content 7
10.4.3 Expression of results 8
10.4.4 Calculation of true protein content 8
10.4.5 Expression of the true protein content results 8

11 Precision **8**
11.1 Interlaboratory test 8
11.2 Repeatability 8
11.3 Reproducibility 8

12 Test report **9**

Annex A (informative) **Results of a collaborative study — Estimation of precision data of direct and indirect methods for the true protein determination** **10**

iTeh STANDARD PREVIEW
(standards.iteh.ai)
ISO 8968-4:2016
https://standards.iteh.ai/catalog/standards/sist/ad8f8310-0260-4b67-a560-9b1549a4c117/iso-8968-4-2016

© ISO and IDF 2016 – All rights reserved

Bibliography **11**

iTeh STANDARD PREVIEW
(standards.iteh.ai)
ISO 8968-4:2016
https://standards.iteh.ai/catalog/standards/sist/ad8f8310-0260-4b67-a560-9931549a4e11/iso-8968-4-2016

 © ISO and IDF 2016 – All rights reserved

Forewords

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

iTeh STANDARD PREVIEW
(standards.iteh.ai)
ISO 8968-4:2016

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products* and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

This second edition of ISO 8968-4|IDF 20-4 cancels and replaces ISO 8968-4|IDF 20-4:2001 and ISO 8968-5|IDF 20-5:2001, which have been technically revised.

ISO 8968|IDF 20 consists of the following parts, under the general title *Milk and milk products — Determination of nitrogen content*:

— *Part 1: Kjeldahl principle and crude protein calculation*

— *Part 3: Block-digestion method (Semi-micro rapid routine method)*[1)]

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

1) It is intended that upon revision, the main element of the title of ISO 8968-3|IDF 20-3 (i.e. "Milk") will be aligned with the main element of the titles of ISO 8968-1|IDF 20-1 and ISO 8968-4|IDF 20-4.

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute and endorsement

ISO 8968-4|IDF 20-4 was prepared by the IDF Standing Committee on *Analytical methods for composition* and ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*.

The work was carried out by the IDF/ISO Project Group (C13) of the Standing Committee on *Analytical methods for composition* under the aegis of its project leaders, D. Barbano (US) and P. Trossat (FR).

This ISO|IDF International Standard cancels and replaces ISO 8968-4|IDF 20-4:2001 and ISO 8968-5|IDF 20-5:2001, which have been technically revised.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 8968|IDF 20 consists of the following parts, under the general title *Milk and milk products — Determination of nitrogen content*:

- *Part 1: Kjeldahl principle and crude protein calculation*
- *Part 3: Block-digestion method (Semi-micro rapid routine method)*
- *Part 4: Determination of protein and non-protein nitrogen content and true protein content calculation (Reference method)*

ISO 8968-4:2016
https://standards.iteh.ai/catalog/standards/sist/ad8f8310-0260-4b67-a560-9931549a4e11/iso-8968-4-2016

© ISO and IDF 2016 – All rights reserved

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)

WARNING — The use of this part of ISO 8968|IDF 20 can involve the use of hazardous materials, operations and equipment. This part of ISO 8968|IDF 20 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.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8968-4:2016
https://standards.iteh.ai/catalog/standards/sist/ad8f8310-0260-4b09-a360-9931549a4e11/iso-8968-4-2016

ISO 8968-1|IDF 20-1, *Milk and milk products — Determination of nitrogen content — Part 1: Kjeldahl principle and crude protein calculation*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
non-protein nitrogen content
NPN
mass fraction of substances determined by the specified procedure

Note 1 to entry: The non-protein nitrogen content is expressed as a percentage by mass.

3.2
protein nitrogen content
mass fraction of substances determined by the specified procedure, directly or, alternatively, indirectly

Note 1 to entry: The protein nitrogen content is 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

© ISO and IDF 2016 – All rights reserved

milk protein by filtration, with the remaining filtrate containing the non-protein nitrogen components. Determination of the nitrogen content of the filtrate by the procedure described in ISO 8968-1|IDF 20-1.

Where the total nitrogen content of the milk sample has previously been determined, the true protein nitrogen content may 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 of the sample.) Determination of the nitrogen content of the precipitate by the procedure described in ISO 8968-1|IDF 20-1.

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 ISO 8968-1 | IDF 20-1, together with the following, are required.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

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.

ISO 8968-4:2016
https://standards.iteh.ai/catalog/standards/sist/ad8f8310-0260-4b67-a560-9931549a4e11/iso-8968-4-2016

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 solution is as described in ISO 8968-1| IDF 20-1.

For the indirect protein nitrogen approach, the following hydrochloric acid solution is required c(HCl) = (0,01 ± 0,000 1) mol/l in addition to the 0,1 mol/l hydrochloric acid solution required as described in ISO 8968-1|IDF 20-1.

6 Apparatus

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

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

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

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

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

© ISO and IDF 2016 – All rights reserved

6.5 Filter paper, nitrogen free, of diameter 15 cm.[2)]

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

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

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|IDF 50.

A representative sample should have been sent to the laboratory. It should not have 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 (6.1). Cool the sample to room temperature while gently mixing the test sample immediately prior to weighing the test portion (9.1 or 10.2.1).

9 Procedure — Direct protein nitrogen approach

9.1 Test portion

iTeh STANDARD PREVIEW
(standards.iteh.ai)

Pipette 5,0 ml ± 0,1 ml of the prepared test sample (see Clause 8) either into a dry and clean Kjeldahl flask or digestion tube, 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.

ISO 8968-4:2016
https://standards.iteh.ai/catalog/standards/sist/ad8f8310-0260-4b67-a560-9931549a4e11/iso-8968-4-2016

NOTE The use of either a Kjeldahl flask or a digestion tube is dependent on the laboratory's choice of digestion apparatus.

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 (6.5) placed in a filter funnel (6.4). 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 (6.6), 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 previously collected 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.

---

2) Whatman No 1 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 and endorsement by ISO or IDF of the product named. Equivalent products may be used it they can be shown to lead to the same results.

© ISO and IDF 2016 – All rights reserved