

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) (ISO 8968-4:2016)

## EESTI STANDARDI EESSÕNA

## NATIONAL FOREWORD

|   |  |
|---|--|
| See Eesti standard EVS-EN ISO 8968-4:2016 sisaldab Euroopa standardi EN ISO 8968-4:2016 ingliskeelset teksti.       | This Estonian standard EVS-EN ISO 8968-4:2016 consists of the English text of the European standard EN ISO 8968-4:2016.            |
| Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas.  | This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation. |
| Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 20.05.2016. | Date of Availability of the European standard is 20.05.2016.   |
| Standard on kättesaadav Eesti Standardikeskusest.   | The standard is available from the Estonian Centre for Standardisation.  |

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile [standardiosakond@evs.ee](mailto:standardiosakond@evs.ee).

ICS 67.100.10

Standardite reprodutseerimise ja levitamise õigus kuulub Eesti Standardikeskusele

Andmete paljundamine, taastekitamine, kopeerimine, salvestamine elektroonsesse süsteemi või edastamine ükskõik millises vormis või millisel teel ilma Eesti Standardikeskuse kirjaliku loata on keelatud.

Kui Teil on küsimusi standardite autorikaitse kohta, võtke palun ühendust Eesti Standardikeskusega:

Aru 10, 10317 Tallinn, Eesti; koduleht [www.evs.ee](http://www.evs.ee); telefon 605 5050; e-post [info@evs.ee](mailto:info@evs.ee)

The right to reproduce and distribute standards belongs to the Estonian Centre for Standardisation

No part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying, without a written permission from the Estonian Centre for Standardisation.

If you have any questions about copyright, please contact Estonian Centre for Standardisation:

Aru 10, 10317 Tallinn, Estonia; homepage [www.evs.ee](http://www.evs.ee); phone +372 605 5050; e-mail [info@evs.ee](mailto:info@evs.ee)

EUROPEAN STANDARD

**EN ISO 8968-4**

NORME EUROPÉENNE

EUROPÄISCHE NORM

April 2016

ICS 67.100.10

Supersedes EN ISO 8968-4:2001, EN ISO 8968-5:2001

English Version

**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) (ISO 8968-4:2016)**

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)

Milch und Milcherzeugnisse - Bestimmung des Stickstoffgehaltes - Teil 4: Bestimmung des Proteinstickstoff-Gehaltes und des Nichtproteinstickstoff-Gehaltes und Berechnung des tatsächlichen Proteingehaltes (Referenzverfahren) (ISO 8968-4:2016)

This European Standard was approved by CEN on 19 March 2016.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

**CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels**

## European foreword

This document (EN ISO 8968-4:2016) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 278 "Milk and milk products - Methods of sampling and analysis" the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2016, and conflicting national standards shall be withdrawn at the latest by October 2016.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 8968-4:2001 and EN ISO 8968-5:2001.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

### Endorsement notice

The text of ISO 8968-4:2016 has been approved by CEN as EN ISO 8968-4:2016 without any modification.

# Contents

Page

|  |           |
|--|-----------|
| Forewords .....  | v         |
| <b>1 Scope .....</b>   | <b>1</b>  |
| <b>2 Normative references .....</b>  | <b>1</b>  |
| <b>3 Terms and definitions .....</b>   | <b>1</b>  |
| <b>4 Principle .....</b>   | <b>1</b>  |
| 4.1 Indirect protein nitrogen .....  | 1         |
| 4.2 Direct protein nitrogen .....  | 2         |
| <b>5 Reagents .....</b>  | <b>2</b>  |
| 5.1 General .....  | 2         |
| 5.2 Trichloroacetic acid (CCl <sub>3</sub> COOH) solution .....  | 2         |
| 5.3 Hydrochloric acid standard volumetric solution .....   | 2         |
| <b>6 Apparatus .....</b>   | <b>2</b>  |
| <b>7 Sampling .....</b>  | <b>3</b>  |
| <b>8 Preparation of test sample .....</b>  | <b>3</b>  |
| <b>9 Procedure — Direct protein nitrogen approach .....</b>  | <b>3</b>  |
| 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         |
| <b>10 Procedure — Indirect protein nitrogen approach .....</b>   | <b>6</b>  |
| 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         |
| <b>11 Precision .....</b>  | <b>8</b>  |
| 11.1 Interlaboratory test .....  | 8         |
| 11.2 Repeatability .....   | 8         |
| 11.3 Reproducibility .....   | 8         |
| <b>12 Test report .....</b>  | <b>9</b>  |
| <b>Annex A (informative) Results of a collaborative study — Estimation of precision data of direct and indirect methods for the true protein determination .....</b> | <b>10</b> |

**Bibliography** ..... **11**

This document is a preview generated by EVS

## 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](http://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](http://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](#)

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)<sup>1)</sup>*
- *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](http://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.

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)*

# 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.

### 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 8968-1|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