
**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 de la teneur en protéines vraies (Méthode de
référence)*



This document is a preview generated by EBS



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

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 ₃ COOH) 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

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).

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](#)

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.

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