

INTERNATIONAL
STANDARD

ISO
17997-1

IDF
29-1

First edition
2004-09-15

**Milk — Determination of casein-nitrogen
content —**

**Part 1:
Indirect method (Reference method)**

*Lait — Détermination de la teneur en azote de caséine —
Partie 1: Méthode indirecte (Méthode de référence)*
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Reference numbers
ISO 17997-1:2004(E)
IDF 29-1:2004(E)

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Published in Switzerland

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 17997-1|IDF 29-1 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 17997|IDF 29 consists of the following parts, under the general title *Milk — Determination of casein-nitrogen content*:

- *Part 1: Indirect method (Reference method)* [ISO 17997-1:2004
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- *Part 2: Direct method*

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 IDF National Committees casting a vote.

ISO 17797-1|IDF 29-1 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 on *Nitrogen compounds*, of the Standing Committee on *Main components in milk*.

This edition of ISO 17997-1|IDF 29-1, together with ISO 17997-2|IDF 29-2, cancels and replaces the first edition of IDF 29:1964, which has been technically revised.

ISO 17997|IDF 29 consists of the following parts, under the general title *Milk — Determination of casein-nitrogen content*:

- *Part 1: Indirect method (Reference method)* [ISO 17997-1:2004](#)
- *Part 2: Direct method* <https://standards.iteh.ai/catalog/standards/sist/24ff7965-1506-417d-9ef8-b8ee92d077ef/iso-17997-1-2004>

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Introduction

This part of ISO 17997|IDF 29 is a classical reference method for the indirect determination of the casein-nitrogen content of milk. No collaborative study data were available for this method when publishing the first edition of IDF 29:1964.

Recent research has been completed to develop a better defined indirect reference method. A routine method for the direct measurement of the casein-nitrogen content of milk is given in ISO 17997-2|IDF 29-2. Both parts of ISO 17997|IDF 29 have been collaboratively studied and a reference to the obtained precision data is now included in each part.

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Milk — Determination of casein-nitrogen content —

Part 1: Indirect method (Reference method)

WARNING — The use of the method and equipment described in this standard may involve 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 health practices and determine the applicability of local regulatory limitations prior to use.

1 Scope

This part of ISO 17997|IDF 29 specifies a reference method for the indirect determination of the casein-nitrogen content of bovine milk.

The method can be modified for milk from other species or liquid dairy products.

NOTE Casein nitrogen will decrease with milk storage time due to casein breakdown even at 4 °C. The casein nitrogen of heat-treated milk will be artificially high because of whey-protein denaturation.

2 Normative references

[ISO 17997-1:2004](https://standards.iteh.ai/catalog/standards/sist/24ff7965-1506-417d-9ef8-b8ee92d077ef/iso-17997-1-2004)

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

ISO 648:1977, *Laboratory glassware — One-mark pipettes*

ISO 1042:1998, *Laboratory glassware — One-mark volumetric flasks*

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

ISO 8968-2|IDF 20-2, *Milk — Determination of nitrogen content — Part 2: Block-digestion method (Macro method)*

3 Terms and definitions

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

3.1

non-casein-nitrogen content

mass fraction of substances determined according to the procedures specified in this part of ISO 17997|IDF 29

3.2 casein-nitrogen content
mass fraction of substances determined according to the procedures specified in this part of ISO 17997|IDF 29

NOTE Both the non-casein-nitrogen content and the casein-nitrogen content are expressed as a mass fraction in percent.

4 Principle

The total-nitrogen content of a test sample is determined by the method of either ISO 8968-1|IDF 20-1 or ISO 8968-2|IDF 20-2. Casein is precipitated from a separate test portion of the same milk by the addition of acetic acid and sodium acetate solutions, such that the final pH of the mixture is approximately 4,6. The precipitated milk casein is removed by filtration, so the remaining filtrate contains the non-casein-nitrogen components. The nitrogen content of the filtrate is determined by the procedure described in ISO 8968-1|IDF 20-1 or ISO 8968-2|IDF 20-2. The casein-nitrogen content is calculated as the difference between the total-nitrogen content and the non-casein-nitrogen content of the milk.

5 Reagents

Use only reagents of recognized analytical grade and glass-distilled water or water of at least equivalent purity.

5.1 Reagents for determination of total nitrogen.

Use the reagents specified in ISO 8968-1|IDF 20-1 or ISO 8968-2|IDF 20-2.

5.2 Acetic acid solution, $c(\text{CH}_3\text{CO}_2\text{H}) = 1,75 \text{ mol/l}$.

Using a volumetric pipette (6.6), add 10,0 ml of glacial acetic acid in a 100 ml volumetric flask (6.3). Dilute to the mark with water.

5.3 Sodium acetate solution, $c(\text{CH}_3\text{CO}_2\text{Na}) = 1 \text{ mol/l}$.

Dissolve 8,20 g of sodium acetate or 13,60 g of sodium acetate trihydrate in water in a 100 ml volumetric flask (6.3). Dilute to the mark with water.

The sodium acetate solution may be stored at room temperature for one week or at between 0 °C and 4 °C for 6 months.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Apparatus for determination of total nitrogen.

Use that specified in ISO 8968-1|IDF 20-1 or ISO 8968-2|IDF 20-2.

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

6.3 One-mark volumetric flasks, with stoppers, of capacity 100 ml, conforming to ISO 1042:1998, class A.

6.4 Bottle-top dispenser, capable of delivering 75 ml of water (optional).

6.5 Graduated measuring cylinder, of capacity up to 100 ml.

6.6 One-mark volumetric pipettes, of capacity 1 ml, 10 ml and 50 ml, conforming to ISO 648:1977, class A.

6.7 Filter funnel, made of glass or plastic, of diameter 75 mm.

6.8 Filter paper, of diameter 15 cm, nitrogen-free (e.g. Whatman No. 1¹⁾ or equivalent).

Pleat before use.

6.9 Conical flasks, or equivalent, of capacity 100 ml.

6.10 Analytical balance, capable of weighing to the nearest 0,1 mg.

6.11 Automatic pipettor or (adjustable) micropipette, capable of delivering 1,0 ml (optional).

7 Sampling

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

Sampling is not part of the method specified in this part of ISO 17997|IDF 29. A recommended sampling method is given in ISO 707.

8 Preparation of test sample

Warm the test sample in the water bath (6.2) set at between 38 °C and 40 °C to melt the milk fat so that a representative test portion of milk can be removed from the test sample. Gently mix the sample immediately prior to removal and weighing of the test portion (see 9.1 and 9.2).

9 Procedure

9.1 Determination of total nitrogen

Determine the total-nitrogen content of the test sample (Clause 8), w_N , expressed as a mass fraction in percent, by using the procedure as described in ISO 8968-1|IDF 20-1 or ISO 8968-2|IDF 20-2.

9.2 Determination of non-casein nitrogen

9.2.1 Test portion

9.2.1.1 Weigh, to the nearest 0,1 mg, approximately 10 g of the prepared test sample (Clause 8) into a 100 ml volumetric flask (6.3). Immediately add 75 ml of water, preheated to 38 °C, using a bottle-top dispenser (6.4) or a measuring cylinder (6.5).

Additional samples may be weighed and water added at this point. However, care shall be taken to finish step 9.2.1.2 within 30 min after adding the test portion to the volumetric flask.

NOTE This 30-min time limit is to minimize proteolytic degradation of casein during sample preparation.

1) Whatman No. 1 is an example of a product available commercially.

This information is given for the convenience of users of this part of ISO 17997|IDF 29 and does not constitute an endorsement by ISO or IDF of this product.