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IDF 29-1

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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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Foreword

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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)*

— *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)*

— *Part 2: Direct method*

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.

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

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, $c(CH_3CO_2H)$ = 1,75 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, $c(CH_3CO_2Na)$ = 1 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. 1[1)] 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.

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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.

9.2.1.2 Using a volumetric pipette (6.6) or an automatic pipettor or (adjustable) micropipette (6.11), add 1 ml of acetic acid solution (5.2) to the flask. Stopper the flask and swirl to mix the contents. Place the flask for 10 min in the water bath (6.2) set at between 38 °C and 40 °C.

Using another volumetric pipette (6.6) or an automatic pipettor or (adjustable) micropipette (6.11), add 1 ml of sodium acetate solution (5.3) to the flask and swirl to mix. Cool the flask and its contents to 20 °C. Dilute to the mark with water at 20 °C. Stopper the flask and invert to mix.

Let the precipitate settle. Filter the contents of the flask using a filter funnel (6.7) and a pleated filter paper (6.8). Collect the entire filtrate in a 100 ml conical flask (6.9). The filtrate shall be clear and free of particulate matter. If not, repeat the procedure starting from 9.2.1 with a new test portion.

NOTE This method uses a fixed volume addition of acetic acid and sodium acetate solutions for every sample. This will not achieve an exact pH of 4,6 for every milk sample. However, it is a practical compromise that has been used traditionally for analysis of bovine milks, particularly when a large number of samples are to be analysed. pH variation in the range between 4,5 and 5,0 has been shown to have a negligible influence on the final result (see Reference [8]). The alternative is to monitor the pH as acid is added to the sample with the appropriate temperature adjustments for pH-meter calibration. In this case, the exact dilution of each test portion should be measured and a different dilution factor should be taken into account for each test portion analysed.

9.2.1.3 Swirl the clear filtrate to ensure it is mixed well. Add 50 ml of filtrate with a volumetric pipette (6.6) to a Kjeldahl flask or digestion tube containing boiling aids, potassium sulfate and copper sulfate solution, as specified in ISO 8968-1|IDF 20-1 or ISO 8968-2|IDF 20-2.

9.2.1.4 Add the appropriate amount of sulfuric acid to the Kjeldahl flask or digestion tube. Digest, distil and titrate as specified for milk in ISO 8968-1|IDF 20-1 or ISO 8968-2|IDF 20-2. When using ISO 8968-2|IDF 20-2, take care to apply gradual heating if the digest foams excessively. The total digestion time may be longer than for the determination of the total-nitrogen content of milk.

The flask or tube may be stoppered after the addition of sulfuric acid. The procedure for digestion, distillation and titration may be carried out at a later time. Record the volume of the titrant, and the hydrochloric acid standard volumetric solution specified in ISO 8968-1|IDF 20-1 used for the titration to at least the nearest 0,05 ml.

9.3 Blank test

Simultaneously with the determination of the test sample, carry out a blank test using the same procedure as described in 9.2.1.2 to 9.2.1.4, but omitting the test portion.

10 Calculation and expression of results

10.1 Calculation

10.1.1 Non-casein-nitrogen content

Calculate the non-casein-nitrogen content of the test sample, w_{NCN}, expressed as a mass fraction in percent, using the following equation:

$$w_{NCN} = \frac{1{,}4007\left(V_s - V_b\right) \times M \times 2 \times 0{,}994}{m}$$

where

V_s is the volume, in millilitres, of the hydrochloric acid used in the determination (9.2.1.4);

V_b is the volume, in millilitres, of the hydrochloric acid used in the determination for the blank test (9.3);

M is the numerical value of the molarity of the hydrochloric acid standard volumetric solution as used in either ISO 8968-1|IDF 20-1 or ISO 8968-2|IDF 20-2;

m is the mass, in grams, of the test portion (9.2.1.1);

0,994 is the multiplication factor, based on the assumption that milk contains a mass fraction of about 3,7 % fat and 2,6 % casein [thus $f = 1 - [(0{,}11 \times 0{,}037) + (0{,}07 \times 0{,}026)] = 0{,}994$ (see Note)].

NOTE The multiplication factor may need to be adjusted if liquid dairy products of significantly different composition or milk from other species are analysed as follows (see Reference [6]):

$f = 1 - [(0{,}11 \times \%\ \text{fat}/100) + (0{,}07 \times \%\ \text{casein}/100)]$

10.1.2 Non-casein-protein content

Calculate the non-casein-protein content of the sample, w_{NCP}, expressed as a mass fraction in percent, using the following equation:

$$w_{NCP} = w_{NCN} \times 6{,}38$$

where 6,38 is the generally accepted multiplication factor to convert the nitrogen content of dairy products to protein content.

10.1.3 Casein-nitrogen content

Calculate the casein-nitrogen content of the test sample, w_{CN}, expressed as a mass fraction in percent, using the following equation:

$$w_{CN} = w_N - w_{NCN}$$

where w_N is the nitrogen content of the test sample, expressed as a mass fraction in percent.

10.1.4 Casein content

Calculate the casein content of the test sample, w_C, expressed as a mass fraction in percent, using the following equation:

$$w_C = w_{CN} \times 6{,}38$$

where 6,38 is the generally accepted multiplication factor to convert the nitrogen content of dairy products to protein content.

10.2 Expression of results

Express the test results to three decimal places if needed for further calculations. In the case of end results, express those obtained for nitrogen content to three decimal places, and for casein content to two decimal places. Do not round the results further until the final use of the test value is made.

NOTE One example is when individual test values from the analysis of many sample materials are going to be used to calculate method performance statistics for within and between laboratory variation. Another example is when the values are to be used as a reference for instrument calibration (e.g. infrared milk analyser) where the values from many samples will be used in a simple or multiple regression calculation.

11 Precision

11.1 Interlaboratory test

The values for repeatability and reproducibility limits were derived from the results of an interlaboratory test carried out in accordance with ISO 5725[2)].

Details of the interlaboratory test of the method are summarized in Reference [5]. The values derived from this test may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

11.2.1 Non-casein nitrogen

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than

— a mass fraction of nitrogen of: 0,004 %, or

— a mass fraction of casein of: 0,03 %.

11.2.2 Casein nitrogen

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than

— a mass fraction of nitrogen of: 0,006 %, or

— a mass fraction of casein of: 0,04 %.

11.3 Reproducibility

11.3.1 Non-casein nitrogen

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than

— a mass fraction of nitrogen of: 0,007 %, or

— a mass fraction of casein of: 0,05 %.

11.3.2 Casein nitrogen

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than

— a mass fraction of nitrogen of: 0,010 %, or

— a mass fraction of casein of: 0,06 %.

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2) ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests* (now withdrawn), was used to obtain the precision data.