INTERNATIONAL STANDARD

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IDF 29-2

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Milk — Determination of casein-nitrogen content —

Part 2: Direct method

Lait — Détermination de la teneur en azote de caséine —

Partie 1: Méthode directe

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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 17997-2 | IDF 29-2 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.

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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 first edition of ISO 17997-2|IDF 29-2, together with ISO 17997-1|IDF 29-1, 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 specifies a routine method for the direct determination of the casein-nitrogen content of milk. The method was developed and optimized to provide results that agree well with results of the method described in ISO 17997-1|IDF 29-1 when both are applied to fresh raw bovine milk.

No collaborative study data were available for this method when publishing its equivalent, IDF 29:1964. Recent research has been completed to develop the better defined indirect reference method given in ISO 17997-1|IDF 29-1, and this routine method for the direct measurement of the casein-nitrogen content of milk. Both methods have been collaboratively studied and a reference to the obtained precision data is included in each part.

Milk — Determination of casein-nitrogen content —

Part 2:
Direct 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 routine method for the direct 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 term and definition applies.

3.1
casein-nitrogen content
mass fraction of substances determined according to the procedures specified in this part of ISO 17997|IDF 29

NOTE The casein-nitrogen content is expressed as a mass fraction in percent.

4 Principle

Casein is precipitated from a test portion of 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 separated by filtration. The filtrate will contain the non-casein-nitrogen components. The nitrogen content of the precipitate is determined by the procedure described in either ISO 8968-1|IDF 20-1 or ISO 8968-2|IDF 20-2.

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 (6.5), 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.

5.4 Buffer solution.

Using a volumetric pipette (6.5), add 1,0 ml of acetic acid solution (5.2) to a 100 ml volumetric flask (6.3). Using another volumetric pipette, add 1,0 ml of sodium acetate solution (5.3) to the flask. Dilute to the mark with water.

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 dispensers, capable of delivering 70 ml of water and 30 ml of buffer solution (5.4), respectively.

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

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

6.7 Filter paper, of diameter 15 cm, nitrogen-free (e.g. Whatman No. 1[1)] or equivalent).

Pleat before use.

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

6.9 Automatic pipettor or **adjustable micropipette**, capable of delivering 0,75 ml.

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 sample. Gently mix the sample immediately prior to removal and weighing of the test portion (9.1).

9 Procedure

9.1 Test portion

Weigh, to the nearest 0,1 mg, approximately 5 g of the prepared test sample (Clause 8) directly into a Kjeldahl flask or digestion tube (6.1) or determine the difference between the mass of a small container containing approximately 5 g of test sample and the mass of the container after the test portion is poured into the flask or tube. Immediately add 70 ml of water, preheated to 38 °C, using the bottle-top dispenser (6.4), while rinsing down any test sample remaining on the inside neck into the bottom of the flask or tube.

Additional test samples may be weighed and water added at this point. However, care shall be taken to finish step 9.2.2 within 30 min after adding the test portion into the Kjeldahl flask or digestion tube.

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

9.2 Determination

9.2.1 Using an automatic pipettor or adjustable micropipette (6.9), add 0,75 ml of acetic acid solution (5.2) to the Kjeldahl flask or digestion tube (9.1), taking care to avoid getting acetic acid solution on the neck of the flask or the tube. Swirl to mix and allow to stand at room temperature for 10 min.

Using an automatic pipettor or adjustable micropipette (6.9), add 0,75 ml of sodium acetate solution (5.3) to the Kjeldahl flask or digestion tube. Again swirl to mix and let the precipitate settle for 10 min. Pour the contents of the Kjeldahl flask or digestion tube through a filter funnel (6.6) containing pleated filter paper (6.7) to collect the filtrate. Some of the precipitate will remain in the Kjeldahl flask or digestion tube and some on the filter paper. It is not necessary to remove all the precipitate from the flask or tube. Filter completely before the next pour.

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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.

Immediately after pouring the mixture, add 30 ml of buffer solution (5.4) with the bottle top dispenser (6.4) to the Kjeldahl flask or digestion tube, taking care not to allow any precipitate to dry on the neck of the Kjeldahl flask or digestion tube. Also use the buffer solution to rinse any precipitate from the neck of the flask or tube down into the bottom. Swirl to mix the contents.

After completion of the filtration of the first pour, pour the buffer rinse from the flask or tube through the same filter paper. Collect this second filtrate by adding it to the first one. Use the same side of the neck of the flask for all pours to reduce the area to which precipitate can adhere.

Again, immediately rinse the neck of the flask or tube with an additional 30 ml of buffer solution (5.4), using the bottle-top dispenser (6.4). Swirl to mix the contents. After completion of the filtration of the first buffer rinse (second pour), pour the second buffer rinse from the flask or tube through the same filter paper, adding the third filtrate to the first two.

The thus-obtained filtrate shall be clear and free of particulate matter. If particles are present, recycle the filtrate through the same filter paper or repeat the whole procedure from 9.1 onwards.

NOTE 1 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.

NOTE 2 At this point, the filtrate is no longer needed and may be discarded in an appropriate manner.

9.2.2 After the filter paper has dried slightly, carefully remove it from the filter funnel and fold the paper to enclose the precipitate. Ensure that no precipitate on the filter paper is lost.

If any precipitate remains on either the inner or outer lip of the Kjeldahl flask or digestion tube, wipe with the folded filter paper so that any precipitate adheres to the paper and then drop the filter paper into the Kjeldahl flask or digestion tube.

9.2.3 Add the boiling aids, the potassium sulfate, the copper solution and the appropriate amount of sulfuric acid to the Kjeldahl flask or digestion tube as specified in ISO 8968-1|IDF 20-1 or ISO 8968-2|IDF 20-2.

Digest, distill and titrate as specified in ISO 8968-1|IDF 20-1 or ISO 8968-2|IDF 20-2. The flask or tube may be stoppered after the addition of sulfuric acid. The digestion, distillation and titration may take place later.

9.3 Blank test

Simultaneously with the determination of the test sample, carry out a blank test using the same procedure as described in 9.1 and 9.2.1 to 9.2.3, but omitting the test portion.

10 Calculation and expression of results

10.1 Calculation

10.1.1 Casein-nitrogen content

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

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

where

V_s is the volume, in millilitres, of the hydrochloric acid used in the determination (9.2.3);

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 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 (9.1).

10.1.2 Casein content

Calculate the casein content, 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 the nitrogen content to three decimal places, and for the 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

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: 0,007 %, or

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

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

11.3 Reproducibility

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: 0,011 %, or

— a mass fraction of casein of: 0,07 %.

12 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the method used, together with reference to this part of ISO 17997|IDF 29;

d) all operating details not specified in this part of ISO 17997|IDF 29, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained and, if the repeatability has been checked, the final quoted result obtained.

Bibliography

[1] ISO 707, *Milk and milk products — Methods of sampling*[3)]

[2] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[3] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[4] ISO 17997-1|IDF 29-1, *Milk — Determination of casein-nitrogen content — Part 1: Indirect method (Reference method)*

[5] *AOAC INTERNATIONAL Compendium of Methods*, 16th ed. (1999 revision), methods 998.05 - 998.07

[6] LYNCH, J.M., BARBANO, D.M. and FLEMING, J.R. *J. Assoc. Off. Anal. Chem. International*, **81**, 1998, pp. 763-774

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3) Equivalent to IDF 50.

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