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Caseins — Determination of free acidity (Reference method)

Caséines — Détermination de l'acidité libre (Méthode de référence)

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org
Web www.fil-idf.org

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Foreword

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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 5547|IDF 91 was prepared by Technical Committee 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 5547|IDF 91 cancels and replaces the first edition (ISO 5547:1978), of which it constitutes a minor revision.

Foreword

IDF (the International Dairy Federation) is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented at the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO 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 the IDF National Committees casting a vote.

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ISO 5547|IDF 91 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by the former Joint ISO-IDF-AOAC Group of Experts (E11-E701) which is now part of the Joint ISO-IDF Action Team on *Physical properties and rheological tests*, of the Standing Committee on *Minor components and characterization of physical properties.*

This edition of ISO 5547|IDF 91 cancels and replaces IDF 91A:1979, of which it constitutes a minor revision.

Caseins — Determination of free acidity (Reference method)

1 Scope

This International Standard specifies a reference method for the determination of the free acidity of caseins obtained by acid precipitation or lactic fermentation and of rennet caseins.

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 385, *Laboratory glassware — Burettes*

ISO 648, *Laboratory glassware — Single volume pipettes*

ISO 835, *Laboratory glassware — Graduated pipettes*

ISO 3310-1, *Test sieves — Technical requirements and testing — Part 1: Test sieves of metal wire cloth*

ISO 4788, *Laboratory glassware — Graduated measuring cylinders*

ISO 5550 | IDF 78, *Caseins and caseinates — Determination of moisture content (Reference method)*

3 Terms and definitions

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

3.1
free acidity of caseins
volume, in millilitres, of a 0,1 mol/l standard volumetric sodium hydroxide solution required to titrate an aqueous extract of 1 g of the product

4 Principle

A test portion at 60 °C is extracted using water and filtered. The filtrate is then titrated against a standard volumetric sodium hydroxide solution, using phenolphthalein as indicator.

5 Reagents

Use only reagents of recognized analytical grade, and only distilled or demineralized water or water of equivalent purity, freed from carbon dioxide by boiling for 10 min before use.

5.1 Sodium hydroxide (NaOH), approximately 0,1 mol/l standard volumetric solution.

5.2 Phenolphthalein ($C_{20}H_{14}O_4$), 10 g/l ethanolic solution.

6 Apparatus

Usual laboratory apparatus, and in particular the following.

6.1 Analytical balance, capable of weighing to the nearest 0,01 g.

6.2 Conical flask, of capacity 500 ml, with ground neck and fitted with a ground-glass stopper.

6.3 One-mark pipette, of capacity 100 ml, complying with the requirements of ISO 648, class A.

6.4 Pipette, suitable for measuring 0,5 ml of indicator solution (5.2), complying with the requirements of ISO 648, class A or ISO 835, class A.

6.5 Conical flask, of capacity 250 ml.

6.6 Measuring cylinder, of capacity 250 ml, complying with the requirements of ISO 4788, class A.

6.7 Burette, graduated in at least 0,1 ml divisions, complying with the requirements of ISO 385, class A.

6.8 Water bath, capable of being maintained at a temperature of (60 ± 2) °C.

6.9 Appropriate filter.

6.10 Grinding device, for grinding the laboratory sample, if necessary (see 8.1.4), without development of undue heat and without loss or absorption of moisture. A hammer-mill shall not be used.

6.11 Test sieve, wire cloth, of diameter 200 mm, with nominal size of aperture 500 µm, with receiver, complying with ISO 3310-1.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707|IDF 50 [1].

8 Procedure

8.1 Preparation of the test sample

8.1.1 Thoroughly mix the laboratory sample by repeatedly shaking and inverting the container (if necessary, after having transferred all of the laboratory sample to an airtight container of sufficient capacity to allow this operation to be carried out).

8.1.2 Transfer about 50 g of the thoroughly mixed laboratory sample to the test sieve (6.11).

8.1.3 If the 50 g portion directly passes or almost completely passes the sieve, use for the determination the sample as prepared in 8.1.1.

8.1.4 Otherwise, grind the 50 g portion, using the grinding device (6.10), until it passes the sieve. Immediately transfer all of the sieved sample to an airtight container of sufficient capacity and mix thoroughly by repeatedly shaking and inverting. During these operations, take precautions to avoid any change in the water content of the product.

8.1.5 After the test sample has been prepared, the determination (8.3) should proceed as soon as possible.

Clean the device after grinding each sample.

8.2 Test portion

Weigh about 10 g of the test sample (8.1) to the nearest 10 mg and transfer it to the conical flask (6.2).

8.3 Determination

Using the 250 ml measuring cylinder (6.6), add 200 ml of freshly boiled water, previously heated to 60 °C. Stopper the flask, mix by swirling and place in the water bath at 60 °C (6.8) for 30 min. Shake the flask at intervals of about 10 min.

Filter, and cool the filtrate to about 20 °C. The filtrate must be clear.

Transfer 100 ml of the cooled filtrate into the conical flask (6.5), using the pipette (6.3). Add 0,5 ml of phenolphthalein solution (5.2), using the pipette (6.4). Titrate with sodium hydroxide solution (5.1), until the appearance of a faint pink colour, persisting for at least 30 s. Record the volume used to the nearest 0,01 ml.

9 Expression of results

9.1 Calculation

9.1.1 The free acidity of the casein, V_{fac}, in millilitres, is given by Equation (1)

$$V_{fac} = \frac{20\, V_{NaOH} c}{m} \qquad (1)$$

where

V_{NaOH} is the volume, in millilitres, of the standard volumetric sodium hydroxide solution (5.1) used;

c is the amount of substance concentration, in moles per litre, of the standard volumetric sodium hydroxide solution (5.1);

m is the mass, in grams, of the test portion.

Calculate the free acidity to the nearest 0,01 ml and report the final result to the nearest 0,1 ml.

9.1.2 To calculate the free acidity of the sample on the dry basis, multiply the result obtained from Equation (1) by Factor (2)

$$\frac{100}{100 - w_w} \qquad (2)$$

where w_w is the water content, as a percentage by mass, of the sample determined according to ISO 5550 | IDF 78.

9.2 Precision

9.2.1 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 0,02 ml of 0,1 mol/l sodium hydroxide solution per 1 g of product.

9.2.2 Reproducibility

The absolute difference between two independent 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 0,04 ml of 0,1 mol/l sodium hydroxide solution per 1 g of product.

10 Test report

The test report shall specify:

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

b) the sampling method used, if known;

c) the test method used, together with a reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incident that may have influenced the result(s);

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