INTERNATIONAL STANDARD

ISO 5548

IDF 106

Second edition
2004-12-01

Caseins and caseinates — Determination of lactose content — Photometric method

Caséines et caséinates — Détermination de la teneur en lactose — Méthode photométrique

Reference numbers
ISO 5548:2004(E)
IDF 106:2004(E)

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Published in Switzerland

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Foreword

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ISO 5548|IDF 106 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.

This edition of ISO 5548|IDF 106 cancels and replaces ISO 5548:1980, of which it constitutes a minor revision. Only editorial changes have been made.

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 the National Committees casting a vote.

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All work was carried out by the Joint ISO/IDF/AOAC Group of Experts, *Methods for caseins and caseinates* (E36), under the aegis of its project leader, Mr J. Eisses (NL).

This edition of ISO 5548|IDF 106 cancels and replaces IDF 106:1982. Only editorial changes have been made.

Caseins and caseinates — Determination of lactose content — Photometric method

1 Scope

This International Standard specifies a photometric method for the determination of the content of lactose and other soluble carbohydrates in caseins and caseinates containing less than 2,0 % of total soluble carbohydrates.

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

3 Terms and definitions

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

3.1
lactose content of caseins and caseinates
content of total soluble carbohydrates, expressed as anhydrous lactose, determined by the procedure specified in this International Standard

NOTE It is expressed as a mass fraction in percent.

4 Principle

A test portion is dissolved

a) in hot water in the case of caseinates;

b) in hot water with the addition of sodium hydrogen carbonate in the case of acid caseins;

c) in hot water with the addition of pentasodium triphosphate in the case of rennet casein.

The casein is precipitated with a solution of acetic acid and sodium acetate at pH 4,6, then filtered to obtain a protein-free solution of the carbohydrates. Phenol solution and concentrated sulfuric acid are added to an aliquot portion of the filtrate, thus producing a colour which is proportional to the amount of carbohydrate present, which is measured photometrically at a wavelength of 490 nm.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

5.1 Sodium hydrogen carbonate ($NaHCO_3$), for analysis of acid casein.

5.2 Pentasodium triphosphate ($Na_5P_3O_{10}$), for analysis of rennet casein.

5.3 Dilute hydrochloric or **sulfuric acid**, c(HCl) or c(1/2 H_2SO_4) = 0,1 mol/l.

5.4 Dilute acetic acid, c(CH_3CO_2H) = 100 g/l.

5.5 Sodium acetate solution, c(CH_3COONa) = 1 mol/l.

5.6 Phenol solution, 80 % (mass fraction).

Heat a mixture of 8 g of phenol and 2 g of water until the mixture is homogeneous.

5.7 Sulfuric acid, concentrated, ρ_{20} (H_2SO_4)= 1,84 g/ml.

5.8 Lactose standard solution, ρ_{20}($C_{12}H_{22}O_{11}$) = 20 g/l.

Weigh 2,105 g $\pm$ 0,001 g of lactose monohydrate, corresponding to 2,00 g of anhydrous lactose, into a 100 ml volumetric flask. Dissolve in water, make up to the mark with water and mix well. Store the obtained standard solution at 0 °C.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

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

6.2 Conical flasks, of capacity 100 ml.

6.3 One-mark pipettes, of capacity 1 ml, 2 ml and 10 ml.

6.4 Micropipettes, of capacity 0,2 ml, with 0,001 ml divisions.

6.5 Graduated pipettes, of capacity 25 ml.

6.6 Test tubes, of capacity about 40 ml, with ground necks and fitted with ground glass stoppers.

6.7 Automatic dispenser, capable of dispensing 5 ml of concentrated sulfuric acid within 1 s.

6.8 Water bath, capable of being maintained at 60 °C to 70 °C.

6.9 Photometer, suitable for making measurements at a wavelength of 490 nm, provided with cells of optical path length 1 cm to 2 cm.

6.10 Mixer, suitable for mixing inside the test tubes (6.6), with a stirrer resistant to strong acid.

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

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

6.13 Volumetric flasks, of capacity 100 ml.

6.14 Water bath, capable of being maintained at 20 °C.

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[1].

8 Procedure

8.1 Preparation of 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 (6.12).

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

8.1.4 Otherwise, grind the 50 g portion, using the grinding device (6.11), until it passes through the sieve. Immediately transfer all 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, carry out the determination (8.5) as soon as possible.

8.2 Preparation of a blank solution

Prepare a blank solution containing 0,1 g ± 0,001 g of sodium hydrogen carbonate (5.1) or 0,1 g ± 0,001 g of pentasodium triphosphate (5.2), as appropriate, using the same apparatus, the same reagents in the same amounts, and the same procedure as described in 8.4.2 to 8.5.1 inclusive, but omitting the test portion and omitting those operations in connection with the presence of a test portion.

For the most accurate results, prepare the blank solution, the test solution and the lactose standard working solutions for the calibration graph (see 8.6) simultaneously.

8.3 Test portion

Weigh, to the nearest 1 mg, about 1 g of the test sample (8.1) into a conical flask (6.2).

8.4 Test solution

8.4.1 In the case of acid casein, add 0,1 g ± 0,001 g of the sodium hydrogen carbonate (5.1).

In the case of rennet casein, add 0,1 g ± 0,001 g of the pentasodium triphosphate (5.2).

8.4.2 Add 25 ml of water, place in the water bath (6.8), set at between 60 °C and 70 °C, and mix occasionally by shaking.

8.4.3 When the test portion is completely dissolved (which generally takes 10 min to 15 min), cool the solution and add successively

— 15 ml of water,

— 8 ml of the dilute hydrochloric or sulfuric acid (5.3), and

— 1 ml of the dilute acetic acid (5.4).

Stopper and mix the contents by shaking after each addition.

8.4.4 Leave for 5 min and then add 1 ml of the sodium acetate solution (5.5). Mix by shaking.

8.4.5 Allow the casein precipitate to settle, then filter through a dry filter paper. Discard the first few millilitres of the filtrate.

8.5 Determination

8.5.1 Pipette 2 ml of the filtrate (8.4.5) into a test tube (6.6). Add 0,2 ml of the phenol solution (5.6) by means of a micropipette (6.4), and mix by shaking. Then add from the automatic dispenser (6.7), in less than 1 s, 5 ml of the concentrated sulfuric acid (5.7), directing the stream of acid against the liquid surface rather than against the side of the test tube in order to obtain good mixing. Immediately mix, using the mixer (6.10), and allow to stand for 15 min. Cool for 5 min in the water bath (6.14) set at 20 °C. Wipe the tube and proceed immediately as described in 8.5.2.

8.5.2 Measure (6.9) the absorbance of the solution (8.5.1) at 490 nm using the blank solution (8.2) as reference solution.

8.5.3 If the absorbance is above the upper limit of the calibration graph (see 8.6), repeat steps 8.5.1 and 8.5.2 using 2 ml of a suitable dilution of the filtrate (8.4.5) instead of 2 ml of the filtrate itself.

If such a dilution is made, the equation given in 9.1 shall be modified accordingly.

8.6 Preparation of calibration graph

8.6.1 Pipette 10 ml of the lactose standard solution (5.8) into a 100 ml volumetric flask (6.13) and dilute to the mark with water (solution A); 1 ml of solution A corresponds to 2 mg of anhydrous lactose.

Prepare three lactose standard working solutions by pipetting 1 ml, 2 ml and 3 ml of solution A into three 100 ml volumetric flasks and making up the volumes with water.

The anhydrous lactose concentrations of the lactose standard working solutions obtained are, respectively, 20 µg/ml, 40 µg/ml and 60 µg/ml.

8.6.2 Using four test tubes (6.6), proceed in accordance with 8.5.1 but replace the 2 ml of filtrate respectively by 2 ml of each of the three lactose standard working solutions and by 2 ml of water.

8.6.3 Measure (6.9) the absorbances of the three lactose standard working solutions, using the solution obtained by treatment of the 2 ml of water as the reference solution.

8.6.4 Construct a calibration graph by plotting the absorbances of the lactose standard working solutions against their anhydrous lactose concentrations in micrograms per millilitre. Draw the best-fitting line through the calibration points.

9 Calculation and expression of results

9.1 Calculation

The lactose content of the sample, w_l, expressed as anhydrous lactose as a mass fraction in percent, is given by the equation

$$w_l = \frac{c \times 10^{-6} \times 50}{m} \times 100\ \%$$

where

c is the concentration, in micrograms per millilitre, of anhydrous lactose in the test solution (8.4.5), read from the calibration curve (8.6.4);

m is the mass, in grams, of the test portion (8.3).

9.2 Expression of results

Express the obtained result to two decimal places.

10 Precision

10.1 Repeatability

For lactose contents less than or equal to 0,2 % (mass fraction), the absolute difference[1)] 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 0,03 %.

10.2 Reproducibility

For lactose contents less than or equal to 0,2 % (mass fraction), the absolute difference[1)] 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 0,04 %.

11 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 test method used, with reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test results;

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

1) At higher lactose contents, this difference will be proportionately greater.

Bibliography

[1] ISO 707, *Milk and milk products — Guidance on sampling* [2)]

2) Equivalent to IDF 50.

ICS 67.100.99

Price based on 6 pages