International Standard 5548

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Caseins and caseinates — Determination of lactose content — Photometric method

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

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ISO 5548-1980 (E)

Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 5548 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in March 1979.

It has been approved by the member bodies of the following countries :

Australia	France	New Zealand
Austria	Germany, F. R.	Romania
Belgium	Hungary	South Africa, Rep. of
Bulgaria	India	Spain
Canada	Israel	Thailand
Chile	Kenya	Turkey
Cyprus	Korea, Rep. of	United Kingdom
Czechoslovakia	Libyan Arab Jamahiriya	Yugoslavia
Egypt, Arab Rep. of	Malaysia	
Ethiopia	Netherlands	

No member body expressed disapproval of the document.

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Caseins and caseinates — Determination of lactose content — Photometric method

1 Scope and field of application

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

2 References

ISO/R 707, *Milk and milk products — Sampling.*

ISO 3310/1, *Test sieves — Technical requirements and testing — Part 1 : Metal wire cloth.*

3 Definition

lactose content of caseins and caseinates : The content of total soluble carbohydrates, expressed as anhydrous lactose as a percentage by mass, determined by the procedure described in this International Standard.

4 Principle

Dissolution of a test portion

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.

Precipitation of the casein with acetic acid and sodium acetate solution at pH 4,6, followed by filtration to obtain a protein-free solution of the carbohydrates. Addition of phenol solution and concentrated sulphuric acid to an aliquot portion of the filtrate, thus producing a colour which is proportional to the amount of carbohydrate present, and photometric measurement at a wavelength of 490 nm.

5 Reagents

All reagents shall be of recognized analytical quality. The water used shall be distilled water or water of at least 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 Hydrochloric acid or **sulphuric acid**, c(HCl) or $c(1/2\ H_2SO_4)$ = 0,1 mol/l.

5.4 Acetic acid, 100 g/l solution.

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

5.6 Phenol, 80 % (m/m) solution.

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

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

5.8 Lactose, 20 g/l solution.

Weigh 2,105 ± 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 volume and mix well. Store the solution at 0 °C.

6 Apparatus

Usual laboratory equipment, and in particular :

6.1 Analytical balance.

6.2 Conical flasks, of capacity 100 ml.

6.3 One-mark pipettes, of capacity 1, 2 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 sulphuric acid within 1 s.

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

6.9 Photometer, suitable for making measurements at a wavelength of 490 nm, provided with cells of optical path length 1 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, wire cloth, 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 controlled at 20 °C.

7 Sampling

See ISO/R 707.

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 air-tight 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 for the determination the sample prepared in 8.1.1.

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 air-tight 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 ± 0,001 g of sodium hydrogen carbonate or 0,1 ± 0,001 g of pentasodium triphosphate, 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.

NOTE — For the most accurate results, prepare the blank solution, the test solution and the standard 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 ± 0,001 g of the sodium hydrogen carbonate (5.1).

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

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

8.4.3 When the test portion is completely dissolved — generally this takes 10 to 15 min — cool and add successively :

— 15 ml of water;

— 8 ml of the hydrochloric acid or sulphuric acid solution (5.3);

— 1 ml of the acetic acid solution (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 sulphuric 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) at 20 °C. Wipe the tube and proceed immediately as described in 8.5.2.

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

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.

NOTE — If such a dilution is made, the formula given in 9.1 has to be modified accordingly.

8.6 Preparation of calibration graph

8.6.1 Pipette 10 ml of the lactose 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 standard solutions by pipetting 1, 2 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 standard solutions obtained are respectively 20, 40 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 standard solutions and by 2 ml of water.

8.6.3 Measure the absorbances of the three standard matching solutions using the solution obtained by treatment of the 2 ml of water as the reference liquid.

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

9 Expression of results

9.1 Method of calculation and formula

The lactose content of the sample, expressed as anhydrous lactose as a percentage by mass, is equal to

$$\frac{\frac{c}{10^6} \times 50}{m} \times 100$$

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 Repeatability[1]

For lactose contents less than or equal to 0,2 % (m/m), the difference between two single results found on identical test material by one analyst using the same apparatus within a short time-interval will exceed 0,03 g of lactose per 100 g of product on average not more than once in 20 cases in the normal and correct operation of the method.

9.3 Reproducibility[1]

For lactose contents less than or equal to 0,2 % (m/m), the difference between two single and independent results found by two operators working in different laboratories on identical test material will exceed 0,04 g of lactose per 100 g of product on average not more than once in 20 cases in the normal and correct operation of the method.

10 Test report

The test report shall show the method used and the result obtained. It shall also mention all operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that may have influenced the result.

The report shall include all details necessary for complete identification of the sample.

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

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