INTERNATIONAL STANDARD 5549

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Caseins and caseinates — Determination of protein content (Reference method)

Caséines et caséinates — Détermination de la teneur en protéines (Méthode de référence)

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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 5549 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in September 1976.

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

Australia	Germany	New Zealand
Austria	Ghana	Portugal
Bulgaria	Hungary	Romania
Canada	India	South Africa, Rep. of
Chile	Iran	Spain
Czechoslovakia	Israel	Turkey
Egypt, Arab Rep. of	Korea, Rep. of	Yugoslavia
France	Netherlands	

The member bodies of the following countries expressed disapproval of the document on technical grounds :

Poland
United Kingdom

NOTE – The method specified in this International Standard has been developed jointly with the IDF (International Dairy Federation) and the AOAC (Association of Official Analytical Chemists, U.S.A.). The text as approved by the above organizations will also be published by FAO/WHO (Code of Principles concerning Milk and Milk Products and Associated Standards), by the IDF and by the AOAC (Official Methods of Analysis).

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Caseins and caseinates — Determination of protein content (Reference method)

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a reference method for the determination of the protein content of caseins and caseinates, excluding those containing ammonium caseinate or other ammonium compounds, or other nitrogenous non-protein compounds.

2 REFERENCES

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

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

ISO 5550, *Caseins and caseinates — Determination of water content (Reference method).* [1)]

3 DEFINITION

protein content of caseins and caseinates : The nitrogen content as determined by the procedure described in this International Standard, multiplied by 6,38 and expressed as a percentage by mass.

4 PRINCIPLE

Digestion of a test portion with a mixture of potassium sulphate and sulphuric acid, in the presence of copper(II) sulphate as catalyst, to convert organic nitrogen into ammoniacal nitrogen. Distillation and absorption of the ammonia in boric acid solution. Titration with standard volumetric hydrochloric acid solution. Multiplication of the result by the factor 6,38.

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

5.2 Potassium sulphate, anhydrous (K_2SO_4).

5.3 Copper(II) sulphate pentahydrate ($CuSO_4.5H_2O$).

5.4 Sucrose ($C_{12}H_{22}O_{11}$).

5.5 Boric acid, 40 g/l solution.

5.6 Sodium hydroxide, concentrated aqueous solution, 30 % (*m/m*).

5.7 Hydrochloric acid, approximately 0,1 N standard volumetric solution, standardized against sodium tetraborate decahydrate ($Na_2B_4O_7.10H_2O$) or anhydrous sodium carbonate (Na_2CO_3).

5.8 Mixed indicator

Mix equal volumes of a 2 g/l solution of methyl red in at least 95 % (*V/V*) ethanol and a 1 g/l solution of methylene blue in at least 95 % (*V/V*) ethanol.

6 APPARATUS

6.1 Analytical balance.

6.2 Kjeldahl flask, 500 ml capacity.

6.3 Digestion apparatus to hold the Kjeldahl flask (6.2) in an inclined position and with a heating device which will not heat the part of the flask above the surface of the liquid contents.

6.4 Condenser with straight inner tube.

6.5 Outlet tube with safety bulb connected to the lower end of the condenser (6.4) by a ground glass joint or a rubber tube. If rubber tubing is used, the glass ends must be near one another.

6.6 Splash-head connected to the Kjeldahl flask (6.2) and to the condenser (6.4) by soft, close-fitting rubber stoppers.

6.7 Conical flask, 500 ml capacity.

6.8 Graduated cylinders, 50 ml and 100 ml capacity.

6.9 Burette, 50 ml capacity, graduated in 0,1 ml.

1) At present at the stage of draft.

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6.10 Boiling aids :

6.10.1 *For the digestion* : small pieces of hard porcelain, or glass beads.

6.10.2 *For the distillation* : freshly calcined pieces of pumice.

6.11 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.

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

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 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 (6.11), until it passes 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, the determination should be proceeded with as soon as possible.

8.2 Test for presence of non-protein nitrogen

If the presence of ammonium caseinate or other ammonium compounds is suspected, carry out the following test. Add to 1 g of sample in a small conical flask, 10 ml of water and 100 mg of magnesium oxide. Rinse down any magnesium oxide adhering to the walls and close the flask with a cork stopper, inserting a piece of red litmus paper between the stopper and the neck of the flask. Mix the contents of the flask carefully and heat the flask in a water bath at 60 to 65 °C. If the litmus paper colours blue within 15 min, ammonia is present, and the method is not applicable (see clause 1).

8.3 Blank test

At the same time as the determination of the nitrogen content of the sample, perform a blank determination using 0,5 g of the sucrose (5.4) instead of the test portion, using the same apparatus, the same quantities of all reagents and the same procedure as described in 8.5. If the result of the blank determination exceeds 0,5 ml of 0,1 N acid, the reagents shall be checked and the impure reagent or reagents purified or replaced.

8.4 Test portion

Transfer to the Kjeldahl flask (6.2) 0,3 to 0,4 g of the test sample (8.1), weighed to the nearest 0,1 g.

8.5 Determination

8.5.1 Transfer to the flask a few pieces of porcelain or a few glass beads (6.10.1) and about 15 g of the anhydrous potassium sulphate (5.2).

Add 0,2 g of the copper(II) sulphate (5.3) and wash down the neck of the flask with a little water. Add 20 ml of the concentrated sulphuric acid (5.1). Mix the contents of the flask.

Heat gently on the digestion apparatus (6.3) until any frothing has ceased. Boil gently until the solution is clear and a pale green-blue colour persists. During heating, shake the flask from time to time.

Continue the boiling, regulating the heating so as to condense the vapours in the middle of the flask neck. Continue the heating for 90 min, avoiding local overheating.

Allow to cool to room temperature. Carefully add about 200 ml of water and a few pieces of pumice (6.10.2). Mix and cool again.

8.5.2 Transfer into the conical flask (6.7) 50 ml of the boric acid solution (5.5) and 4 drops of the indicator (5.8). Mix. Place the conical flask under the condenser (6.4) so that the tip of the outlet tube (6.5) is immersed in the boric acid solution. Using a graduated cylinder (6.8), add to the Kjeldahl flask 80 ml of the sodium hydroxide solution (5.6). During this operation, hold the flask in an inclined position so that the sodium hydroxide solution runs down the side of the flask to form a bottom layer.

Immediately connect the Kjeldahl flask to the condenser by means of the splash-head (6.6).

Gently rotate the Kjeldahl flask to mix its contents. Boil gently at first, avoiding any frothing. Continue the distillation so that 150 ml of distillate are collected in approximately 30 min. The distillate should have a temperature below 25 °C. About 2 min before the end of the distillation, lower the conical flask so that the tip of the outlet tube is no longer immersed in the acid solution, and

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rinse the tip with a little water. Stop heating, remove the outlet tube and rinse its outer and inner walls with a little water, collecting the washings in the conical flask.

8.5.3 Titrate the distillate in the conical flask, using the standard volumetric hydrochloric acid solution (5.7).

9 EXPRESSION OF RESULTS

9.1 Method of calculation and formula

9.1.1 The protein content of the sample, expressed as a percentage by mass, is equal to

$$\frac{(V_1 - V_2) \times T \times 1{,}4}{m} \times 6{,}38$$

$$= \frac{8{,}932\,(V_1 - V_2) \times T}{m}$$

where

V_1 is the volume, in millilitres, of the standard volumetric hydrochloric acid solution (5.7) used in the determination (8.4);

V_2 is the volume, in millilitres, of the standard volumetric hydrochloric acid solution (5.7) used in the blank test (8.3);

T is the normality of the standard volumetric hydrochloric acid solution (5.7);

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

Calculate the protein content to the nearest 0,1 %.

9.1.2 To calculate the protein content of the sample on the dry basis, as a percentage by mass, multiply the result obtained in accordance with 9.1.1 by

$$\frac{100}{100 - M}$$

where M is the water content of the sample determined according to ISO 5550.

9.2 Precision

9.2.1 *Repeatability*

The difference between two single results obtained on identical test material by one analyst using the same apparatus within a short time interval will exceed 0,5 g of protein per 100 g of product on average not more than once in 20 cases in the normal and correct operation of the method.

9.2.2 *Reproducibility*

The difference between two single and independent results obtained by two operators working in different laboratories on identical test material will exceed 1,0 g of protein 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.

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