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**Caseins and caseinates — Determination  
of contents of scorched particles and of  
extraneous matter**

*Caséines et caséinates — Détermination de la teneur en particules  
brûlées et en matières exogènes*

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## Foreword

**ISO (the International Organization for Standardization)** is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 5739|IDF 107 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 5739|IDF 107 cancels and replaces the first edition of ISO 5739:1983, which has been technically revised.

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## 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.

ISO 5739|IDF 107 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.

All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Physical properties of dried milk products*, of the Standing Committee on *Minor components and characterization of physical properties*, under the aegis of its project leader, Mr. J. de Vilder (BE).

This edition of ISO 5739|IDF 107 cancels and replaces the first edition of IDF 107A:1995, which has been technically revised.

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## Introduction

The method is derived from procedures developed by the United States Department of Agriculture (USDA) for the determination of scorched particles in dried milk.

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# Caseins and caseinates — Determination of contents of scorched particles and of extraneous matter

## 1 Scope

This International Standard specifies a method for the determination of the contents of scorched particles and of extraneous matter in caseins and caseinates.

## 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

#### **scorched particles content of caseins and caseinates**

amount of coloured residue per 25 g of test sample, determined and classified by the procedure specified in this International Standard

NOTE The scorched particles content is expressed by classification.

### 3.2

#### **extraneous matter**

any foreign matter in the product associated with objectionable conditions or practices in production, storage or distribution

## 4 Principle

A test portion is dissolved in a hot solution of sodium carbonate, sodium polyphosphate or disodium ethylenediaminetetraacetate, or in sodium hydroxide solution at room temperature or at  $60\text{ °C} \pm 1\text{ °C}$ . The solution obtained is filtrated through a filtering disc and dried. The dried disc with the scorched particles is visually compared with standard discs.

## 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 carbonate solution** ( $\text{Na}_2\text{CO}_3$ ), for acid caseins and for caseinates.

Dissolve 100 g of anhydrous sodium carbonate in water in a 1 000 ml conical flask (6.2). Dilute to 1 000 ml with water and mix thoroughly until completely dissolved. Use the filtering device (6.6) and the filtering disc (6.5) to filter the solution into another 1 000 ml conical flask.

**5.2 Sodium polyphosphate solution** ( $\text{Na}_5\text{P}_3\text{O}_{10}$ ), for rennet caseins.

Dissolve 20 g of a sodium polyphosphate (tripolyphosphate or a higher polyphosphate as used in the manufacture of processed cheese) in water in a conical flask (6.2). Dilute to 1 000 ml with water and mix thoroughly until complete dissolution. Use the filtering device (6.6) and the filtering disc (6.5) to filter the solution into another 1 000 ml conical flask.

**5.3 Disodium ethylenediaminetetraacetate solution** ( $\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8$ ), for calcium caseinates.

Dissolve 100 g of disodium ethylenediaminetetraacetate (EDTA) dihydrate in water in a 1 000 ml conical flask (6.2). Dilute to 1 000 ml with water and mix thoroughly until completely dissolved. Use the filtering device (6.6) and the filtering disc (6.5) to filter the solution into another 1 000 ml conical flask.

**5.4 Sodium hydroxide solution** ( $\text{NaOH}$ ),  $c(\text{NaOH}) \approx 1 \text{ mol/l}$ .

Use this sodium hydroxide solution for caseins and caseinates which do not dissolve completely in the other reagents. Dissolve 40 g of sodium hydroxide in water in a 1 000 ml conical flask (6.2). Dilute to 1 000 ml with water and mix thoroughly until completely dissolved. Use the filtering device (6.6) and the filtering disc (6.5) to filter the solution into another 1 000 ml conical flask.

**5.5 Ethanol**, of volume fraction 95 %  $\pm$  2 %, for caseinates.

## 6 Apparatus

Usual laboratory equipment and, in particular, the following.

**6.1 Analytical balance**, capable of weighing to the nearest 0,1 g.

**6.2 Conical flasks**, of capacities 600 ml and 1 000 ml.

**6.3 Measuring cylinders**, of capacities 100 ml and 500 ml.

**6.4 Water bath**, capable of maintaining a temperature of  $60 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$ .

**6.5 Filtering discs**, of diameter 32 mm, suitable for use in the filtering device (6.6).

The filter material should preferably consist of cotton, and the limit for retaining scorched particles and extraneous matter should be 5  $\mu\text{m}$  to 10  $\mu\text{m}$ . In addition, the disc shall be tested by the method specified in Annex A.

**6.6 Filtering device**, aspirator or pressure type, with a filtering area of diameter 28,6 mm.

**6.7 Grinding device**, for grinding the test sample, if necessary.

To avoid loss of moisture, the device should not produce undue heat. A hammer shall not be used.

**6.8 Test sieve**, of metal wire cloth, with diameter 200 mm and nominal aperture size 500  $\mu\text{m}$ , with receiver, complying with the requirements of ISO 3310-1.

**6.9 Scorched particle standard discs**, indicating increasing scorched particles content by the classification letters A, B, C and D, respectively (see Figure 1 for examples).

Instructions for the preparation of the scorched particle standard discs, if required, are given in Annex B.

NOTE Prints of the standard discs are available from the United States Department of Agriculture, Agricultural Marketing Service, Washington DC 20250. Order by name and number<sup>1)</sup>. Figure 1 shows examples of the discs. This illustration is not to be used for official classification of scorched particles content.

**6.10 Microscope**, providing a magnification of  $\times 25$ .

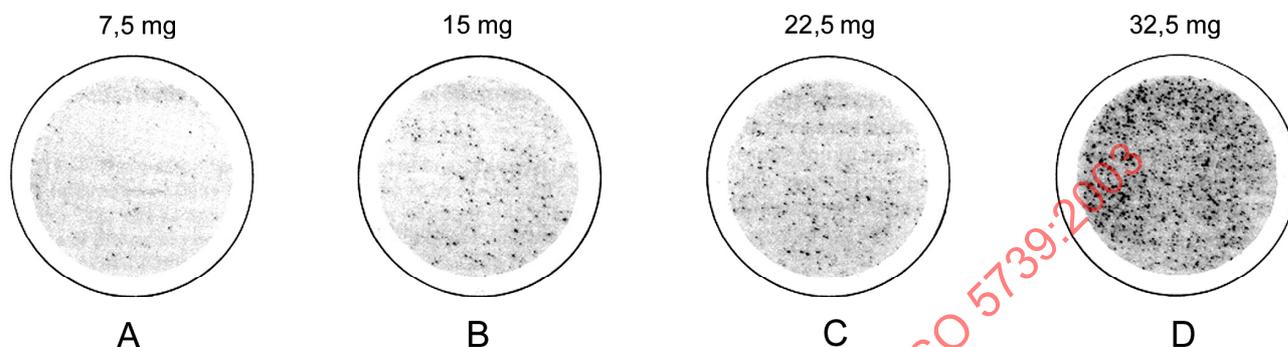


Figure 1 — Examples of standard scorched particle discs

## 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 International Standard. A recommended sampling method is given in ISO 707.

## 8 Procedure

### 8.1 Preparation of test sample

**8.1.1** Thoroughly mix the test sample by repeatedly shaking and inverting the container. If necessary, transfer the complete test 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 test sample to the test sieve (6.8). If the amount of test sample directly passes or almost completely passes the sieve, use the test sample as prepared in 8.1.1. Proceed with the preparation of the test portion (8.2) and the determination (8.3) as soon as possible after the preparation of the sample.

**8.1.3** If the 50 g test sample does not directly pass the sieve, grind the test sample by using the grinding device (6.7) until it passes 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 all precautions to avoid any change in the moisture content of the product. Proceed with the preparation of the test portion (8.2) and the determination (8.3) as soon as possible after the preparation of the test sample.

1) USDA scorched particle standards for dry milks, No. 7 CFR 2858 2676, is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of this product.

## 8.2 Test portion

Weigh, to nearest 0,1 g, 25,0 g of the test sample (8.1.2 or 8.1.3) and transfer the test portion to a 600 ml flask (6.2).

## 8.3 Determination

### 8.3.1 Addition of reagent

NOTE The pH of the solutions obtained in 8.3.1.1 to 8.3.1.3 and those in 8.3.3 a), b), c) might have an effect on the partial solubility of the scorched particles. The solution pH can vary due to possible pH variation of the test samples. It is recommended, therefore, to check and record the pH of the obtained solutions.

#### 8.3.1.1 Caseinates

Add 50 ml of ethanol (5.5) to the test portion (8.2) in the 600 ml conical flask. Shake to moisten the whole test portion and add 300 ml of filtered water. Allow the obtained test solution to stand for at least 10 min.

Swirl at frequent intervals until the test portion is dispersed. Then add 30 ml of the sodium carbonate solution (5.1) and swirl again. Take care to wash down any of the matter adhering to the walls of the flask.

If the test portion consists of calcium caseinates or other insoluble caseinates, add, before the addition of the sodium carbonate solution (5.1), 30 ml of the disodium ethylenediaminetetraacetate solution (5.3). Allow the obtained test solution to stand for at least 10 min.

Swirl at frequent intervals until the test portion is dispersed. Take care to wash down any of the matter adhering to the walls of the flask. Then add the 30 ml of the sodium carbonate solution (5.1) and swirl again.

#### 8.3.1.2 Rennet caseins

Add 250 ml of filtered water to the test portion (8.2) in the conical flask. Shake the obtained test solution and allow to stand for 3 h. Then add 500 ml of the sodium polyphosphate solution (5.2). Take care to wash down any of the matter adhering to the walls of the conical flask.

#### 8.3.1.3 Acid caseins

Proceed as described in 8.3.1.1, omitting the ethanol (5.5) and using 50 ml of sodium carbonate solution (5.1).

### 8.3.2 Heating and filtration

8.3.2.1 Swirl the conical flask with the test solution (8.3.1.1, 8.3.1.2 or 8.3.1.3). Cover the flask and heat the test solution in the water bath (6.4) set at 60 °C. Swirl the flask at frequent intervals until the test portion is completely dissolved. The time required to dissolve the test portion completely shall not exceed 45 min.

8.3.2.2 Swirl the conical flask once more and filter its contents through the filtering disc (6.5) mounted in the filtering device (6.6). Rinse the flask with two successive 100 ml portions of water. Allow the rinsings to run down the walls of the filtering device.

8.3.2.3 If difficulties are encountered in passing the solution through a disc, or if a significant quantity of gelatinous material appears on the disc, repeat the determination by using the procedure specified in 8.3.3 and 8.3.4.

8.3.2.4 Remove the filtering disc and allow it to dry or, alternatively, dry it in a drying oven set at between 30 °C and 40 °C, protected from dust.

### 8.3.3 Addition of alternative reagents

See Note in 8.3.1.

If the test portion dissolves unsatisfactorily according to 8.3.1 and 8.3.2, proceed as follows.

- a) **For acid caseins:** Proceed as specified in 8.3.1.3, but instead of adding 50 ml of the sodium carbonate solution (5.1), add 30 ml of the sodium hydroxide solution (5.4) to the test solution in the conical flask and swirl.
- b) **For rennet caseins:** Add 375 ml of filtered water to the 25 g test portion (8.2) in the conical flask. Swirl the test solution and allow to stand for at least 3 h. Add 37,5 ml of filtered sodium hydroxide solution (5.4) and swirl.
- c) **For caseinates:** Proceed as specified in 8.3.1.1, but instead of adding 30 ml of the sodium carbonate solution (5.1), add 30 ml of the sodium hydroxide solution (5.4) to the test solution in the conical flask and swirl. Do not add disodium ethylenediaminetetraacetate solution (5.3).

### 8.3.4 Heating and filtration when sodium hydroxide solution is added (alternative procedure to 8.3.2)

**8.3.4.1** Cover the contents of the flask [8.3.3 a), b) or c)] and allow to stand at room temperature. Swirl at frequent intervals until the contents of the flask are dissolved completely. Proceed as specified in 8.3.2.2 and 8.3.2.3.

**8.3.4.2** If the contents of the flask are not completely dissolved, or if filtering (8.3.2.2) is difficult, heat the covered flask in the water bath (6.4) set at 60 °C for 20 min. Swirl the flask at frequent intervals until its contents are dissolved. Proceed as specified in 8.3.4.1.

## 9 Evaluation and expression of results

### 9.1 Evaluation

#### 9.1.1 Scorched particles content

Compare the test disc (8.3.2.4) with the scorched particles standard discs (6.9), viewing from directly above in uniform indirect light. Assign the appropriate classification letter to the test disc. Assign a test disc falling between two standard discs to the classification letter corresponding to the higher scorched particles content.

#### 9.1.2 Extraneous matter

Examine the disc for extraneous matter, using a microscope (6.10) at a magnification of  $\times 25$  for identification. Report any extraneous matter by name, number of particles and size.

### 9.2 Expression of results

Express the scorched particles content by classification letters A, B, C or D respectively (for example, see Figure 1).

## 10 Precision

### 10.1 Repeatability

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, shall indicate the same classification.

### 10.2 Reproducibility

Two independent single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, shall indicate the same classification.

## 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, together 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.

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## Annex A (normative)

### Testing of filtering discs

#### A.1 Scope

This annex specifies a method of testing the suitability of filtering discs. It has been derived from reference [2].

#### A.2 Materials

**A.2.1 Wetting agent solution**, with a volume fraction of 1 % wetting agent.

Use an aerosol solution or a solution of any other suitable wetting agent.

**A.2.2 Gum solution.**

Add 0,75 g of carob bean or another suitable gum to 100 ml of water, while stirring in a blender.

**A.2.3 Sucrose solution.**

Dissolve 750 g of sucrose in 750 ml of water.

**A.2.4 Formaldehyde solution** (CH<sub>2</sub>O).

Dissolve 40 ml of pure formaldehyde in 60 ml of water.

**A.2.5 Fine sediment mixture**, 0,2 g/l dispersion.

Prepare the mixture from ground cow manure, dried in the oven (A.3.3) set at 100 °C, garden soil and charcoal. Sieve the materials separately and collect the fractions passing through a sieve of aperture size 106 µm (diameter 203,2 mm) and retained on a sieve of aperture size 75 µm, proceeding as follows.

Place separately the cow manure and the soil (not more than 100 g of each), and not more than 50 g of charcoal, on the sieve of aperture size 106 µm nested over the sieve of aperture size 75 µm. Cover, and position the receiver. Shake the nest of sieves by hand for 5 min at a rate of about 120 strokes per minute.

In maximum batches of about 20 g, sieve the fractions retained again on the 75 µm sieve as above for 5 min. Use the fractions retained from this second sieving operation and mix them uniformly in the following proportions:

- cow manure      66 %;
- garden soil      28 %;
- charcoal          6 %.

Place 2 g of the mixture in a 100 ml volumetric flask (A.3.5) and moisten with 5 ml of the wetting agent solution (A.2.1). Add 46 ml of gum solution (A.2.2). Dilute the liquid level just to the neck of the flask by adding the sucrose solution (A.2.3) and mix.

Allow the solution to stand for at least 30 min. Add a few drops of ethanol (5.5). Dilute to the mark with the sucrose solution (A.2.3) and mix thoroughly.

Remove air bubbles by treating the solution under vacuum or by heating. Boil, cool, and then add 2 ml of the 40 % formaldehyde solution

NOTE The addition of the 40 % formaldehyde solution causes separation of insoluble vegetable fragments and permits the use of clear supernatant solution.

In the absence of a blender, dry stabilizer may be mixed with ethanol (5.5) to facilitate dispersion in water.

Pour the solution into a 250 ml beaker or screw-cap bottle. Stir, allowing minimal incorporation of air, with a small mechanical stirrer at between 200 r/min and 300 r/min until the sediment is uniformly distributed, as observed under a bright reflected light. Position the blade of the stirrer so that the fine particles do not accumulate in small eddies at the bottom of the beaker.

While stirring, transfer a 10 ml portion (corresponding to 200 mg of fine sediment mixture), by means of a graduated pipette (A.3.6), to a 1 000 ml volumetric flask (A.3.5). Dilute to the 1 000 ml mark with water and mix.

### A.3 Apparatus

Usual laboratory equipment and, in particular, the following.

- A.3.1 **Analytical balance**, capable of weighing to the nearest 0,1 mg.
- A.3.2 **Desiccator**, provided with an efficient desiccant.
- A.3.3 **Oven**, capable of maintaining a temperature of  $100\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ .
- A.3.4 **Filter papers**, of diameter 7 cm or 9 cm, high quality and medium grade.
- A.3.5 **One-mark volumetric flasks**, of capacities 100 ml and 1 000 ml.
- A.3.6 **Graduated pipettes**, provided with outlets of diameter about 3 mm.

### A.4 Procedure

A.4.1 Wash a filter paper (A.3.4), placed in a Buchner<sup>2)</sup> funnel, with about 200 ml of water. Dry it to constant mass in the oven (A.3.3) set at  $100\text{ }^{\circ}\text{C}$ . Cool in a covered dish in a desiccator (A.3.2) and weigh. Check on constant mass by repeating the drying, cooling and weighing procedure.

A.4.2 After thoroughly mixing and stirring, filter 60 ml of the fine sediment mixture (A.2.5), corresponding to 12 mg of sediment, through a filtering disc (6.5) mounted in the filtering device (6.6). Use a clean flask to collect the filtrate. Transfer the filtrate to a beaker. Rinse the flask three times with water. Add the rinsings to the contents of the beaker.

A.4.3 Again filter the filtrate through the washed, dried and weighed filter paper (see A.4.1) placed in a Buchner funnel. Rinse the beaker and paper thoroughly with water. Dry the filter paper to constant mass in the oven (A.3.3) set at  $100\text{ }^{\circ}\text{C}$  as specified in A.4.1.

A.4.4 Test at least two more discs.

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2) Buchner is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of this product.

## A.5 Evaluation

The average mass of sediment per disc passing through three or more discs should not exceed 2,8 mg after drying to constant mass.

A standard disc prepared from a fine sediment mixture should not appear to have sediment buried beneath the surface.

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## Annex B (normative)

### Preparation of standard discs of scorched particles

#### B.1 Scope

This annex specifies a procedure for the preparation of standard discs of scorched particles. It has been derived from reference [3].

#### B.2 Materials

##### B.2.1 Sucrose solution.

Dissolve 750 g of sucrose in 750 ml of water in a 1 000 ml conical flask (6.2). Filter the sucrose solution before use.

##### B.2.2 Charcoal mixture, 1,0 g/l dispersion.

Prepare a charcoal mixture of at least 1 g having the following composition (mass fraction):

- 20 % of 200 mesh charcoal;
- 50 % of 150 mesh charcoal;
- 20 % of 100 mesh charcoal;
- 10 % of 65 mesh charcoal.

Transfer 1,0 g of the above charcoal mixture to a 1 000 ml volumetric flask (B.3.4). Dilute to the mark with the sucrose solution (B.2.1) and mix thoroughly.

##### B.2.3 Spray-dried, non-fat milk solids.

##### B.2.4 Reconstituted, non-fat dry milk solids.

Dissolve 50 g of spray-dried non-fat milk solids (B.2.3) in 500 ml of water. Filter before use.

#### B.3 Apparatus

Usual laboratory equipment and, in particular, the following.

##### B.3.1 Analytical balance, capable of weighing to the nearest 0,1 mg.

##### B.3.2 Petri dish.

##### B.3.3 Desiccator, provided with an efficient desiccant.

##### B.3.4 One-mark volumetric flasks, of capacity 200 ml and 1 000 ml respectively.

##### B.3.5 Oven, capable of maintaining a temperature of $119\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ .