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Milk products — Determination of the acidification activity of dairy cultures by continuous pH measurement (CpH)

Produits laitiers — Détermination de l'activité acidifiante des cultures laitières par mesurage continu de pH (CpH)

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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 26323 | IDF 213 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.

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 on 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 26323 | IDF 213 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 ISO-IDF Joint Action Team on *Lactic acid bacteria and starters* of the Standing Committee on *Microbiological methods of analysis* under the aegis of its project leader, Mr. L. V. Jørgensen (DK).

Milk products — Determination of the acidification activity of dairy cultures by continuous pH measurement (CpH)

1 Scope

This International Standard specifies a method for the measurement of the acidification activity of lactic acid bacteria by continuous measurement of pH.

NOTE The method is based on Reference [9].

The method is applicable to dairy starter cultures where these characteristic microorganisms are present.

Two types of standardized milk are specified in the procedure: boiled milk with 9,5 % mass fraction dry matter (B-milk 9,5); and autoclaved milk with 9,5 % mass fraction dry matter (A-milk 9,5). It is possible that heat treatment of B-milk 9,5 does not inactivate all enzymes that are present, which can affect the activity of some cultures. In that case, cultures are tested with A-milk 9,5 in which all enzymes have been inactivated.

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 6887-5, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 5: Specific rules for the preparation of milk and milk products* [1)]

3 Terms and definitions

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

3.1
acidification activity
ability of a dairy starter culture to acidify standardized milk determined according to the procedure described in this International Standard

NOTE The acidification activity can be quantified by the following parameters.

a) t_a — The time it takes to start acidifying the standardized milk, i.e. time for pH to drop 0,08 pH-units from initial pH (after 15 min). Time t_a is measured in minutes from the inoculation time, $t = 0$. If the software collects data every 4 min, t_a is determined by interpolation.

b) $pH_{t\,h}$ — The pH after t h (e.g. 4 h, 6 h, 12 h or 16 h) of acidification in the standardized milk at 30 °C, 37 °C, 40 °C or 43 °C. The actual time for the parameter depends on the characteristics of the starter culture.

c) $t_{pH\,x}$ — The time it takes to acidify the standardized milk to a certain pH, e.g. pH 4,50. The actual time for the parameter depends on the characteristics of the starter culture and the application for which it is used.

1) Supersedes ISO 8261 | IDF 122.

4 Principle

A specified quantity of starter culture is diluted and inoculated in a specified amount of standardized milk. The inoculated culture is incubated at a specified constant temperature of 30 °C, 37 °C, 40 °C or 43 °C for a given time depending on the characteristics of the starter culture. During incubation, the acidification activity is measured by continuous pH measurements using a pH electrode and a data logger. When the fermentation curve is obtained, a number of curve parameters can be calculated or extracted.

5 Diluents, culture media and reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and only distilled or demineralized water or water of equivalent purity. The water shall be free of substances likely to inhibit or influence the growth of microorganisms in the reconstituted milk. If chlorinated water is used, neutralize the chlorine prior to use.

Observe the following rules for the water quality: the cell count shall be below 50 cell/ml and conductivity below 5 µS/cm. Tests to determine the suitability of water for microbiological applications appear in ISO/TS 11133-1 [5].

5.1 Basic materials

SAFETY PRECAUTIONS — Take the required safety precautions when using the electrode cleaner (5.1.2), potassium chloride solution (5.1.3) or diaphragm cleaner (5.1.4) as these can irritate skin and eyes.

5.1.1 Buffer solutions

5.1.1.1 Buffer solution of pH 4,00, capable of buffering at 20 °C; e.g. Merck/VWR CertiPUR (Order No. 1.09475) [2] or equivalent.

5.1.1.2 Buffer solution of pH 7,00, capable of buffering at 20 °C; e.g. Merck/VWR CertiPUR (Order No. 1.09477) [2] or equivalent.

5.1.2 Electrode cleaner, capable of cleaning the electrode; e.g. pepsin/HCl solution from Mettler Toledo (Order no. 9891) [2] or equivalent.

5.1.3 Potassium chloride solution, of concentration c(KCl) = 3 mol/l; e.g. Mettler Toledo (Order no. 9823) [2] or equivalent.

5.1.4 Diaphragm cleaner, capable of cleaning the diaphragm; e.g. thiourea/HCl solution from Mettler Toledo (Order no. 9892)[2] or equivalent.

5.1.5 Ethanol solution, φ (C_2H_5OH) = 70 % volume fraction in water, used for disinfection.

5.1.6 Medium-heat, low fat, spray-dried milk powder. Use medium-heat, low fat, spray-dried milk powder produced from milk of good quality and with no detectable antibiotics residues. The spray-dried milk powder shall be of good microbiological quality (see Reference [8]) having a natural, pleasant taste and flavour of fresh skimmed milk and shall consist of the components specified in Table 1.

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2) 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 either ISO or IDF of this product. Other products may be used if they can be shown to give similar results.

Table 1 — Medium-heat, low fat, spray-dried milk powder compound[a], e.g. from Chr. Hansen[3)]

Component	Mass fraction %
Milk protein	34 to 38
Lactose	48 to 56
Milk fat	< 1,25
Ash	7 to 9
Moisture	<4
Titratable acidity, lactic acid	< 0,15
[a] The pH of a 10 % mass fraction solution should be in the range 6,5 to 6,8.	

5.2 Substrates

5.2.1 QC-Milk 9,5

5.2.1.1 Composition. Use only standardized milk with a specified dry matter content prepared from spray-dried skimmed milk powder according to the composition in Table 2.

The prepared amount may be smaller than that specified in Table 2. However, do not prepare less than ~1,1 kg of milk. If using a small amount, use the analytical balance (6.1) to weigh the milk powder to the nearest 1 mg.

The aim is to prepare milk with a dry matter content of 9,5 % ± 0,2 % mass fraction, similar to that of bottled and sterilized milk.

Table 2 — Composition of milk made up from spray-dried milk powder

Component	Mass kg
Medium-heat milk powder [a]	10,9 to 11,2
Water	100,0
[a] The quantity varies due to water content of milk powder and water loss during the preparation and heat treatment.	

5.2.1.2 Preparation. Dissolve the medium-heat milk powder in water for not more than 30 min. If needed, warm the water up to 40 °C to completely dissolve the powder.

Dispense the milk obtained into cylindrical bottles to reach a volume of 200 ml after heat treatment. Check the volume by weighing the bottle until it reaches a net mass of 207 g ± 2 g (total mass minus that of the bottle). The density of QC-milk 9,5 is 1,033 g/ml.

Alternatively, dispense the milk obtained into cylindrical bottles to reach a mass of 200 g ± 2 g (net mass). Compensate for the mass difference in the inoculation procedure.

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3) 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 either ISO or IDF of this product. Other products may be used if they can be shown to give similar results.

5.2.2 Heat treatment of B-milk 9,5. Heat treat the prepared milk (5.2.1.2) according to the temperature programme in Table 3.

Table 3 — Temperature programme for heat treating B-milk 9,5

Procedure	Temperature °C	Time min
Heating	> 99 ± 1	< 20
Holding	99 ± 1	30 ± 1
Cooling	99 ± 1 to 40 ± 5	< 40

Then, store the B-milk 9,5 obtained at below 7 °C for at least 16 h.

Record the evaporation during heat treatment and the tolerance of mass after the heat treatment.

5.2.3 Shelf life of B-milk 9,5. Before use, store the B-milk 9,5 (5.2.2) for a minimum of 16 h and a maximum of 12 d.

Enzymes such as proteases may not have been inactivated and can affect acidification activity of some cultures.

5.2.4 Heat treatment of A-milk 9,5. Use A-milk 9,5 for cultures where B-milk 9,5 does not give the required repeatability, e.g. due to residual protease activity.

Sterilize the prepared milk (5.2.1.2) according to the temperature programme in Table 4.

Table 4 — Temperature programme for heat treating A-milk 9,5

Procedure	Temperature °C	Time min
Heating	115 ± 1	< 20
Holding	115 ± 1	15 ± 1
Cooling	115 ± 1 to 40 ± 5	< 40

Then, store the A-milk 9,5 obtained at below 7 °C for at least 16 h.

Record the evaporation during heat treatment and the tolerance of mass after that treatment.

5.2.5 Shelf life of A-milk 9,5. Before use, store the A-milk 9,5 (5.2.4) for a minimum of 16 h and a maximum of 12 d.

6 Apparatus

Usual microbiological laboratory equipment and, in particular, the equipment required for the preparation of test samples and dilutions specified in ISO 6887-5, as well as the following.

6.1 Analytical balances, capable of weighing to the nearest 0,01 g and 1 mg (see 5.2.1.1), respectively.

6.2 Autoclave, capable of operating at 99 °C ± 1 °C and 115 °C ± 1 °C.

6.3 Water baths, capable of being maintained at 21 °C ± 1 °C, 30,0 °C ± 0,2 °C, 37,0 °C ± 0,2 °C, 40,0 °C ± 0,2 °C and 43,0 °C ± 0,2 °C, under thermostat control.

6.4 pH electrodes, suitable for measuring the required pH; e.g. Mettler Toledo [4)] 405-DPAS-SC-K8S/150 or equivalent electrodes.

6.5 Cylindrical bottles, capacity 250 ml, of height 16,5 cm and internal diameter 5,5 cm.

6.6 Temperature probes, calibration of accuracy ± 0,1 °C.

6.7 Data logger, equipped with pH and temperature channels; connected to a computer capable of logging data from pH and temperature probes.

7 Sampling

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

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

8 Preparation

8.1 Milk preparation

At least 16 h before starting the analysis, loosen the lids of the milk bottles (second weighing) to be used in the activity analysis. Allow oxygen and carbon dioxide concentrations to reach equilibrium between milk and surroundings.

Allow the water baths to pre-warm to the temperature of inoculation.

Cool the milk bottles used for dilution (first weighing) to between 4 °C and 12 °C to minimize the microbial activity.

Pre-warm the milk bottles for activity analysis (second or third weighing, see 9.1.2) to the temperature of inoculation in the water bath at the required temperature for 20 min to 40 min. The total time depends on the incubation temperature and may not exceed 60 min prior to inoculation.

Set the temperature for continuous measurement by a calibrated temperature probe in a control bottle. Check the temperature before inoculation. Alternatively, calibrated thermometers can be used.

8.2 Cleaning and calibration of pH electrodes

Warm the buffer solution of pH 4,00 (5.1.1.1), the buffer solution of pH 7,00 (5.1.1.2), and the pH electrodes (6.4) to the incubation temperature of the water bath (6.3) maintained at 30,0 °C ± 0,2 °C, 37,0 °C ± 0,2 °C, 40,0 °C ± 0,2 °C or 43,0 °C ± 0,2 °C for at least 10 min before calibrating the pH probes.

Use the pH-value of the buffers at a temperature of 30 °C, 37 °C, 40 °C or 43 °C as supplied by the manufacturer when calibrating the pH probes.

NOTE The pH values of the buffers are temperature dependent and, on request, can be obtained from the manufacturer or supplier.

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