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Milk — Determination of freezing point — Thermistor cryoscope method (Reference method)

Lait — Détermination du point de congélation — Méthode au cryoscope à thermistance (Méthode de référence)

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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 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 5764|IDF 108 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 joint edition of ISO 5764|IDF 108 cancels and replaces the first edition of ISO 5764:1987, which has been technically revised.

Annexes A to C of this International Standard are for information only.

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 National Committees casting a vote.

International Standard ISO 5764|IDF 108 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, *Water*, of the Standing Committee on *Main components of milk*, under the aegis of its project leader, Mr H.J.C.M. van den Bijgaart (NL).

This joint edition of ISO 5764|IDF 108 cancels and replaces the second edition of IDF 108:1991, which has been technically revised.

Introduction

The method described in this International Standard for the determination of the freezing point of milk uses a thermistor cryoscope, in which a thermostatically controlled device is cooled and a thermistor probe is used for the measurement of the freezing point.

This reference method requires the use of plateau-timed instruments. For routine measurements, other thermistor cryoscope methods, i.e. fixed time procedures, can be used. Guidelines for the application of other procedures are given in annex B.

Milk — Determination of freezing point — Thermistor cryoscope method (Reference method)

1 Scope

This International Standard specifies a reference method for the determination of the freezing point of raw, pasteurized, UHT-treated or sterilized whole milk, partially skimmed milk and skimmed milk by using a thermistor cryoscope.

The freezing point can be used for estimating the proportion of extraneous water in milk. Calculation of the amount of extraneous water is complicated by daily variation, seasonal variation, etc. and is not within the scope of this International Standard.

Results obtained from samples with a titratable acidity exceeding 20 ml of 0,1 mol/l sodium hydroxide solution per 10 g of non-fat solids will not be representative of the original milk.

NOTE Sterilization and vacuum pasteurization can affect the freezing point of milk (see reference [6]).

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 6091, *Dried milk — Determination of titratable acidity (Reference method)*

3 Term and definition

For the purposes of this International Standard, the following term and definition applies.

3.1
freezing point of milk
value obtained using the method specified in this International Standard

NOTE The freezing point is expressed in millidegrees Celsius (m°C).

4 Principle

A test sample of milk is super-cooled to an appropriate temperature and crystallization is induced by means sufficient to cause an instantaneous release of heat with an accompanying warming of the sample to a temperature

plateau. The plateau is reached when the temperature rise has not exceeded 0,5 m°C over the last 20 s. The thus-attained temperature corresponds to the freezing point of the milk sample.

The instrument is calibrated by adjusting it to give the correct readings for two sodium chloride standard solutions, using the same procedure as for test portions of milk.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

5.1 Water, complying with grade 2 in accordance with ISO 3696, or water of equivalent quality distilled from borosilicate glass apparatus.

The water shall be boiled and cooled to 20 °C ± 2 °C shortly before use.

5.2 Sodium chloride (NaCl), finely ground, dried in the electric furnace (6.7) at 300 °C ± 25 °C for 5 h or alternatively dried in the drying oven (6.8) at 130 °C ± 2 °C for at least 24 h, then cooled to room temperature in a desiccator (6.9).

5.3 Sodium chloride standard solutions

Weigh, to the nearest 0,1 mg, the appropriate amount (see Table 1) of prepared dry sodium chloride (5.2) in a weighing bottle (6.5). Dissolve in water (5.1) and transfer quantitatively to a 1 000 ml one-mark volumetric flask (6.6). Dilute to the 1 000 ml mark with water (5.1) at 20 °C ± 2 °C and mix.

Alternatively and preferably, make up the sodium chloride standard solution on a gram per kilogram basis (see Table 1, second column) by weighing the required amount of prepared dry sodium chloride (5.2) to the nearest 0,1 mg and dissolving it in exactly 1 kg of water (5.1). Store standard solutions at about 5 °C in well-stoppered polyethylene bottles (6.10) of capacity not greater than 250 ml.

Before using a standard solution, gently invert and rotate the bottle several times to mix its contents thoroughly. At no time should the standard solution be agitated violently, as this can lead to incorporation of air. Pour samples of standard solutions from the bottles. Never use pipettes for this purpose. Do not use standard solutions from bottles less than one-quarter full, or more than 2 months old, or containing visible moulds.

Table 1 — Freezing point of sodium chloride standard solutions

NaCl solution at 20 °C g/l solution	**NaCl solution** g/kg water	**Freezing point** m°C
6,731	6,763	− 400,0
6,868	6,901	− 408,0
7,587	7,625	− 450,0
8,444	8,489	− 500,0
8,615	8,662	− 510,0
8,650	8,697	− 512,0
8,787	8,835	− 520,0
8,959	9,008	− 530,0
9,130	9,181	− 540,0
9,302	9,354	− 550,0
9,422	9,475	− 557,0
10,161	10,220	− 600,0

For this reference method, only unpreserved sodium chloride standard solutions should be used. For routine methods, sodium chloride standard solutions with a fungicidal or fungistatic agent may be used. For guidance, see annex B.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Cryoscope, consisting of a thermostatically controlled cooling device, a thermistor probe with associated circuit, a read-out device, a sample agitator and a crystallization device (see Figure 1).

6.1.1 Cooling device

Several types of thermostated cooling devices may be used. The following types are given as examples:

— immersion type: a cooling bath with a suitable buffer capacity;

— circulation type: a continuous stream of cooling liquid around the sample tube;

— cooling block type: a cooling block with a small amount of cooling liquid.

After the initiation of freezing, keep the temperature of the cooling liquid around the sample tube constant at − 7,0 °C ± 0,5 °C.

NOTE A suitable cooling liquid is a 33 % (volume fraction) aqueous solution of propylene glycol.

6.1.2 Measuring device, associated circuitry and read-out device

The thermistor shall be of the glass-probe type with diameter of 1,60 mm ± 0,4 mm and an electrical resistance of between 3 Ω and 30 kΩ at 0 °C.

The type and dimensions of the shank material (including a possible filler) shall not allow a heat transfer into the sample greater than $2{,}5 \times 10^{-3}$ J/s, under operating conditions.

When the probe is in the measurement position, the thermistor bead shall lie on the axis of the sample and at equal distances from the inner walls and the inner bottom of the tube (see Figure 1).

The thermistor and the associated circuitry shall show a discrimination of 1 m°C or better over a range of −400 m°C to −600 m°C.

The linearity of the circuit shall be such that no error greater than 1 m°C is introduced at any point within the range of −400 m°C to −600 m°C when the instrument is correctly operated.

The read-out device shall provide a discrimination of 1 m°C or better over a range of at least 0 m°C to −1 000 m°C.

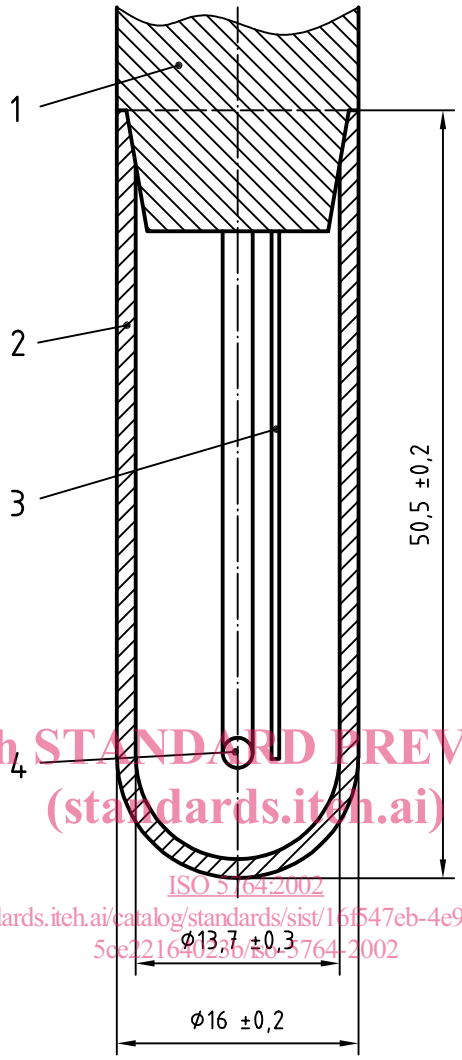
6.1.3 Stirring wire, inert to milk, used to stir the test portion during cooling.

The stirring wire shall be adjustable for amplitude and be mounted vertically in accordance with the manufacturer's instruction. The wire shall vibrate laterally with an amplitude of 2 mm to 3 mm to ensure that the temperature within the test portion remains uniform during cooling. At no time during its normal stirring operation shall the wire strike the glass probe or the wall of the tube.

6.1.4 Device for initiating freezing: any device that, when operated, instantaneously initiates freezing of the test portion when reaching −3,0 °C.

The stirring wire (6.1.3) may be used for this purpose. One method is to increase the amplitude of vibration for 1 s to 2 s such that the stirring wire strikes the wall of the sample tube (6.2).

Dimensions in millimetres



Key

1	Mandrel	3	Stirring wire
2	Sample tube	4	Thermistor bead

Figure 1 — Detail of thermistor cryoscope

6.2 Sample tubes, symmetrical, made of borosilicate glass, of length 50,5 mm ± 0,2 mm, external diameter 16,0 mm ± 0,2 mm, and internal diameter 13,7 mm ± 0,3 mm (see Figure 1).

The wall thickness throughout the tube shall not vary by more than 0,1 mm.

The tubes shall be equally shaped so that equal freezing points are obtained for equal volumes of the same solution. Check on equality before using the tubes.

6.3 Main power supply, capable of operating within the manufacturer's specifications.

6.4 Analytical balance, capable of weighing to the nearest 0,1 mg.

6.5 Weighing bottle

6.6 One-mark volumetric flasks, of capacity 1 000 ml, complying with the requirements of ISO 1042, class A.

6.7 Electric furnace, capable of being controlled at 300 °C ± 25 °C.

6.8 Drying oven, capable of being controlled at 130 °C ± 2 °C.

6.9 Desiccator

6.10 Polyethylene bottles, of maximum capacity 250 ml, with a suitable stopper.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707.

It is important that the laboratory receive a sample that is truly representative and has not been damaged or changed during transport or storage.

If necessary, the test samples may be stored at a temperature of between 0 °C and 6 °C.

It is preferable to test the samples immediately upon arrival at the laboratory.

8 Calibration of the thermistor cryoscope

Ensure that the cryoscope (6.1) is in working condition in accordance with the manufacturer's instructions. Check the position of the probe, the amplitude of vibration of the stirring wire and the temperature of the cooling device (6.1.1).

Select two sodium chloride standard solutions (see Table 1) which closely bracket the expected value of the freezing point of the milk to be tested. The difference in the freezing points between the two selected sodium chloride standard solutions shall not be less than 100 m°C. Take care that the temperatures of the selected sodium chloride standard solutions and that of the test sample are similar.

Pour 2,5 ml ± 0,1 ml of the sodium chloride standard solutions into clean, dry sample tubes (6.2) and calibrate the instrument as indicated by the manufacturer. Use sample tubes (6.2) of the same type as those being used during testing of the sample. Thereafter, the thermistor cryoscope is ready for use.

9 Preparation of test sample

9.1 Preparation

If necessary, remove any visible foreign bodies or solid butterfat from the test sample by filtering into a clean dry vessel. Mix the sample gently. Use a filter that is inert to milk and effective when used at laboratory temperature.

Test the samples at their storage temperature or after having reached the laboratory temperature before commencing the determination. However, the test samples and the sodium chloride standard solutions should have the same temperature when commencing the determination (see also clause 8).

9.2 Sample condition

If doubts exist about the sample condition, determine the titratable acidity of the test sample by the method specified in ISO 6091 as far as possible at the same time as determining the freezing point.

Results obtained from test samples with a titratable acidity exceeding 20 ml of 0,1 mol/l sodium hydroxide solution per 10 g of non-fat solids will not be representative of the original milk.