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Milk — Determination of freezing point — Thermistor cryoscope method

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

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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.

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. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 5764 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

NOTE — The method specified in this International Standard has been developed jointly with the International Dairy Federation (IDF) and the Association of Official Analytical Chemists (AOAC) and will also be published by these organizations.

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

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Milk — Determination of freezing point — Thermistor cryoscope method

0 Introduction

The method described in this International Standard for the determination of the freezing point of milk uses an apparatus (the thermistor cryoscope) in which a thermostatically controlled bath is cooled by electrical refrigeration and a thermistor probe replaces the mercury-in-glass thermometer used in the classical Hortvet method [1].

There are two types of instrument available. One is an instrument that seeks the maximum freezing point on the "plateau" in the freezing curve, while the second, for commercial reasons, is set to read at a fixed time after the onset of freezing. As freezing point curves may differ from milk to milk and between milk and the standard solutions used for calibration, this reference method requires the use of plateau-seeking instruments [2]. Fixed-time instruments may be used for routine screening measurements.

1 Scope and field of application

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

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

The method is applicable to whole milk, partially skimmed milk and skimmed milk, whether raw or pasteurized, UHT-treated, homogenized or sterilized. Sterilization and vacuum pasteurization can, however, affect the freezing point of milk [2] and results obtained from samples with an acidity exceeding 0,18 g of lactic acid per 100 ml of milk will not be representative of the original milk.

2 References

ISO 707, *Milk and milk products — Methods of sampling.*

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

3 Definition

freezing point of milk: The value measured according to the method specified in this International Standard, and expressed in degrees Celsius (°C).

4 Principle

Supercooling a test portion of the milk to the appropriate temperature, depending on the instrument, and inducement of crystallization by mechanical vibration, which causes the temperature to rise quickly to a plateau which corresponds to the freezing point of the test portion.

The instrument is calibrated by adjusting it to give the correct readings for two standard solutions, using the same procedure as for test portions of milk. In these conditions, the plateau gives the freezing point of the milk in degrees Celsius.

5 Apparatus and materials

NOTE — The components of the thermistor cryoscope specified in this International Standard are those which have been in general use for some time. This enables manufacturers to make the complete apparatus to a standard specification and users to check that the apparatus conforms to it. For the measuring device, the present practice is to use a Wheatstone bridge with galvanometer and measuring dial or a digital voltmeter.

5.1 Cryoscope

The cryoscope consists of a thermostatically controlled cooling bath, thermistor probe (a semi-conductor resistance thermometer) with associated circuit and galvanometer or "read-out", sample agitator and a device for initiating freezing, together with sample tubes.

5.1.1 Cooling bath.

Two types of cooling bath can be used.

NOTE — A suitable cooling liquid is a 33 % (V/V) aqueous solution of 1,2-ethanediol (ethylene glycol).

5.1.1.1 Immersion type

This comprises a well-insulated bath containing a suitable cooling liquid, which is stirred so that the temperature difference between any two points in the liquid does not exceed 0,2 °C.

The temperature of the liquid shall not fluctuate by more than ± 0,5 °C from the nominal value stated by the manufacturer.

It is important that the liquid in the cooling bath be maintained at a constant level. All of the surface of the sample tube below the volume mark shall be covered by the cooling liquid.

5.1.1.2 Circulation type

With this type, a continuous stream of suitable cooling liquid is circulated around the sample tube. The temperature of the liquid shall not fluctuate by more than ± 0,5 °C from the nominal value stated by the manufacturer.

5.1.2 Thermistor and accompanying circuit.

The thermistor shall be of the glass probe type, of diameter not greater than 1,8 ± 0,2 mm and with a lead diameter not greater than 0,31 mm. The time constant of the thermistor shall be less than 2 s and the value of β (see the note) shall be high. The working voltage, current and dissipation constant should be such that the thermistor temperature is not raised by more than 0,000 5 °C above its surroundings at −0,512 °C. The maximum tolerance on the resistance shall be ± 5 %.

When the probe is in the working position in the cryoscope, the tip of the glass bead shall lie in the axis of the sample tube and at a point 44,6 ± 0,1 mm below the top of the tube (see the figure). A template shall be provided to enable the user to set the probe in this position.

NOTE — β defines the resistance/temperature characteristics of the thermistor according to the formula

$$-\frac{dR}{dT}\cdot\frac{1}{R}=\frac{\beta}{T^2}$$

where

T is the temperature, in kelvins;

R is the resistance, in ohms, at temperature T;

$-\frac{dR}{dT}\cdot\frac{1}{R}$ is the temperature coefficient;

β is a constant that depends upon the material used to make the thermistor. In current practice, a value of β in excess of 3 000 is recommended.

5.1.3 Measuring and read-out device.

5.1.3.1 Principle of measurement

The instrument used shall operate on the principle of seeking the first "plateau" in the freezing point curve. The plateau is the part of the curve in which the temperature remains constant to within ± 0,001 °C for a minimum of 20 s.

Devices for manual or automatic operation are available.

5.1.3.2 Manual operation

The resistance of the thermistor shall be balanced by means of a Wheatstone bridge or similar device, using the highest quality stable resistors whose tolerance is not greater than ± 10 % and whose temperature coefficient does not exceed $2 \times 10^{-5}\ °C^{-1}$.

The variable (balancing) resistor shall not depart from linearity over the whole of its range by more than 0,3 % of its maximum value.

There shall be a means of adjusting the resistors for calibration purposes.

The measuring dial shall be graduated at intervals not greater than 0,001 °C.

5.1.3.3 Automatic operation

The read-out device shall provide a discrimination of at least 0,001 °C over the range 0 to −1 °C.

The stability of the read-out device and its associated circuit shall be such that successive indications of the same temperature do not vary by more than 0,001 °C.

The linearity of the circuit shall be such that no error greater than ± 0,001 °C is introduced at any point within the range −0,400 °C to −0,600 °C when the instrument is correctly operated.

5.1.4 Stirring wire.

A wire of metal inert to milk and of diameter between 1 and 1,5 mm is used to stir the test portion.

The stirring wire should be adjusted for amplitude and shall be mounted vertically with its lower end level with the tip of the thermistor probe. A tolerance of about 1,5 mm above this position is permitted but on no account shall the wire extend below the level of the probe.

The wire shall vibrate laterally with sufficient amplitude (usually about ± 1,5 mm) to ensure that the temperature within the test portion remains uniform during the determination. At no time during its normal stirring operation shall the wire strike the thermistor probe or the wall of the tube.

5.1.5 Device for initiating freezing.

This may be any device that, when operated, instantaneously initiates freezing of the test portion so that the temperature of the test portion rises towards the freezing point. The stirring wire may be used for this purpose; one method is to increase the amplitude of vibration for 1 to 2 s so that the stirring wire strikes the wall of the sample tube.

5.1.6 Sample tubes.

The sample tubes (see the figure) shall be made of glass and shall be 50,8 ± 0,1 mm long, 16 ± 0,1 mm in external diameter and 13,5 ± 0,1 mm in internal diameter. The wall thickness throughout the tube shall not vary by more than 0,1 mm.

The tubes should carry a volume mark 29,8 mm below the rim (21 mm above the base of the tube) to indicate a sample volume of 2,5 ± 0,1 ml.

5.1.7 Electricity supply.

The supply voltage shall be stabilized, either within the apparatus or externally, so that fluctuation does not exceed ± 1 % of the nominal value when the mains supply fluctuates by ± 6 %.

5.2 Ancillary apparatus

5.2.1 Analytical balance, accurate to 0,1 mg.

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

5.2.3 Drying oven, well ventilated, capable of being controlled at 130 ± 1 °C, or **electric furnace**, ventilated, capable of being controlled at 300 ± 25 °C.

5.2.4 Desiccator.

5.3 Materials

5.3.1 Water, distilled from borosilicate glass apparatus, and boiled and cooled to 20 ± 2 °C shortly before use.

5.3.2 Sodium chloride, analytical reagent quality, finely ground, previously dried for 5 h at 300 ± 25 °C in the electric furnace (5.2.3) or alternatively dried in the oven (5.2.3) at 130 ± 1 °C for at least 24 h and cooled to room temperature in an efficient desiccator.

5.3.3 Standard solution.

Weigh the appropriate amount (see the table) of the dry sodium chloride (5.3.2) in a weighing bottle. Dissolve in water (5.3.1), transfer quantitatively to a 1 000 ml one-mark volumetric flask, and dilute to the mark with the water at 20 ± 2 °C.

Store at about 5 °C in well-stoppered polyethylene bottles of capacity not greater than 250 ml.

Table — Freezing point of sodium chloride solutions

g NaCl/l	°C	°H*
6,859	−0,408	−0,422
7,818	−0,464	−0,480
8,149	−0,483	−0,500
8,314	−0,492	−0,510
8,480	−0,502	−0,520
8,646	−0,512	−0,530
8,811	−0,521	−0,540
8,977	−0,531	−0,550
9,143	−0,541	−0,560
10,155	−0,600	−0,621

* Nominal °C as measured in Hortvet apparatus. (See also annex B.)

NOTES

1 Before using a standard solution, gently invert and rotate the bottle several times to mix its contents thoroughly. At no time should a standard solution be agitated violently as this may lead to incorporation of air.

2 Samples of a standard solution should be withdrawn from the bottle by pouring; pipettes should never be used for this purpose.

3 Solutions should not be used from bottles less than one quarter full, and, if not preserved with a fungicide (for example thiomersal solution, 10 g/l), should not be used if more than two months old.

6 Calibration of thermistor cryoscope

Ensure that the cryoscope is in working condition in accordance with the manufacturer's instructions, and has been switched on for at least 12 h prior to calibration. Check the position of the probe, the amplitude of vibration of the stirring wire and the temperature of the cooling liquids.

Select two standard solutions (see the table) which closely bracket the expected freezing point value of the milk to be tested. The difference in freezing points between the two solutions should preferably be not less than 0,100 °C.

(In some designs of currently available cryoscopes, the circuit associated with the thermistor is designed to be balanced at a specific value of freezing point within the measuring range of the instrument. In these cases, the use of a standard solution having this freezing point as one of the calibrating solutions facilitates the calibration procedure, and this value shall be indicated by the manufacturer.)

Pour 2,5 ml of one standard solution into a clean, dry sample tube and operate the cryoscope.

NOTE — The sample tubes used during calibration should be made from the same type of glass as those used during testing the milk samples. The temperatures of the standard solutions should be similar to those of the milk samples.

Adjust the calibration controls, as indicated by the manufacturer, until the cryoscope reading is equal to the freezing point of the standard solution. Repeat the procedure with the other standard solution, and continue alternating in this way until successive readings on each solution, without further adjustment of the calibration controls, give the correct value of the freezing point of each. The cryoscope is then ready for use and will indicate directly the freezing point of the milk sample, without the application of any correction.

7 Sampling and preparation of sample

7.1 Sample the milk in accordance with ISO 707.

7.2 It is preferable to test the samples immediately but, if necessary, laboratory samples may be stored at a temperature below 5 °C. Samples may be stored for up to 12 weeks at −18 °C or may be preserved for a shorter period with an isotonic solution of a bacteriostatic agent [2].

Remove any visible foreign bodies or solid butterfat from the sample, if necessary by filtering into a clean dry vessel, and mix the sample gently. The filter, if used, shall be inert to milk and shall be effective when used at laboratory temperature.

7.3 The milk may be tested when it is at its storage temperature (between 0 and 5 °C) or it may be allowed to reach laboratory temperature before commencing the determination.

However, it is desirable that the standard solutions and the milk samples are at the same temperature when used.

7.4 Determine the titratable acidity of the milk by the method specified in ISO 6091, as far as possible at the same time as the determination of the freezing point.

8 Procedure

8.1 Preliminary checks

Check that the level of the cooling liquid is in accordance with the manufacturer's instructions and that, if appropriate, the thermistor probe is in an empty sample tube in the sample well. Switch on the cryoscope and ensure that cooling liquid is being properly stirred or circulated, as appropriate. When the cryoscope has been switched on for at least 12 h, check the temperature of the cooling liquid and the position and amplitude of vibration of the stirring wire.

8.2 Routine calibration check

Before each series of determinations, measure the freezing point of a standard sodium chloride solution (for example the solution having a freezing point of −0,512 °C) until the values obtained in two consecutive determinations do not differ by more than 0,001 °C. If the mean of these values differs from the freezing point of the standard solution by more than 0,002 °C, recalibrate the cryoscope as described in clause 6.

If the cryoscope is in continuous use, carry out the routine calibration check at least once per hour.

8.3 Determination

Gently invert and rotate the sample container several times to mix its contents.

Transfer, by pouring or by means of a pipette, a test portion of 2,5 ± 0,1 ml of the milk into a clean, dry, sample tube. Ensure that the probe and stirring wire are clean and dry, if necessary wiping carefully with a soft, clean, lintless tissue.

Insert the sample tube into the calibrated cryoscope according to the manufacturer's instructions. The milk will be cooled and freezing initiated within 0,1 °C of the temperature specified by the manufacturer.

(On some automatic instruments, this temperature may be observed on the digital read-out; on manual instruments, the required precision is achieved by ensuring that freezing starts when the galvanometer pointer or hair-line coincides with the appropriate mark.)

If, for any reason, freezing is initiated before the specified temperature range, abandon the determination and repeat with another test portion of the milk. If this second test portion also freezes before the specified temperature, warm a portion of the sample to about 45 °C and maintain it at this temperature for 5 min to allow melting of crystalline fat. Then cool again to 20 ± 2 °C and test immediately. The temperature of the milk after initiation of freezing will rise rapidly to a value which will remain virtually constant for some time before falling again. The freezing point corresponds to the highest temperature reached during this period and this value shall be recorded.

NOTE — The time during which the temperature remains constant and the time interval between initiation of freezing and the attainment of the highest temperature will differ from sample to sample and will be considerably shorter for water and standard sodium chloride solutions than for milk. It is essential that it is the highest temperature that is recorded.

When the measurement has been satisfactorily completed, remove the tube, rinse with water and then dry the thermistor probe and stirring wire with a soft, clean, lintless tissue and carry out a second determination on another portion of the milk sample. If the two freezing points differ by more than the repeatability value (0,004 °C), discard the results and carry out a further two consecutive determinations on fresh test portions.

8.4 Cooling of probe

After using the instrument, place an empty sample tube in the sample well and lower the operating head in order to keep the probe cool. (In certain designs of cryoscope, this may not be possible; in this case, it is essential to ensure that the probe is adequately cooled before taking measurements, for example by making several dummy determinations until consistent readings are obtained.)

9 Expression of results

9.1 Mean value

If, following the routine calibration check, the calibration is confirmed, take as the result the mean of the two values obtained, rounded to the third decimal place. (See annex A.)

9.2 Precision

NOTE — The values for repeatability and reproducibility were established by various interlaboratory studies carried out in a number of countries[3] and are expressed for the 95 % probability level.

9.2.1 Repeatability

The difference between the results of two determinations, carried out in rapid succession by the same operator using the same apparatus to determine the freezing point of identical milks, should not exceed 0,004 °C.

9.2.2 Reproducibility

The difference between the results of two determinations, carried out by two different laboratories using this standard method to determine the freezing point of identical milks, should not exceed 0,006 °C.

10 Test report

The test report shall include the following information:

a) reference to this International Standard;

b) full identification of the laboratory sample, including information on storage conditions and preservatives, if used;

c) freezing point;

d) titratable acidity;

e) any observations that suggest that the result may be unreliable;

f) date received and date tested.

Annex A

Mean of duplicate values

If the sum of two acceptable duplicate values is an odd number, the mean should be rounded to the nearest even value as shown in the following examples.

Examples:

Freezing points (°C)		
Duplicate values		**Mean**
−0,544	−0,545	−0,544
−0,545	−0,546	−0,546

Annex B

Relationship between freezing points expressed in degrees Celsius and degrees Celsius as measured in the Hortvet apparatus

Hortvet[1] used a thermometer calibrated by the US Bureau of Standards to determine the freezing point of sucrose solutions of concentrations 70 g/l (−0,422 °C) and 100 g/l (−0,621 °C), so that such solutions could be used as reference points for the calibration of other cryoscope thermometers. It is possible that Hortvet assumed that these figures represented the true freezing points for sucrose solutions of these concentrations, but they actually represent the values obtained when the Hortvet cryoscope and the Hortvet technique are used. The true freezing points are −0,407 46 °C and −0,599 68 °C. This has two consequences:

a) the observed temperature depressions of the sucrose solutions are too large;

b) the interval between them is not 0,199 °C but is 0,192 °C.

Sucrose solutions have now been replaced by sodium chloride solutions with the same freezing points.

It is therefore apparent that all of the results in the literature which have been obtained by the use of cryoscopes based on the Hortvet principle are not true freezing points. Providing the method of calibration of the cryoscope is given, however, it is possible to correct results from one method of determination to the other, as follows.

$$T_C = 0{,}965\,6\ T_H$$

and

$$T_H = 1{,}035\,6\ T_C$$

where

T_C is the freezing point in degrees Celsius;

T_H is the freezing point in degrees Hortvet (°H).

The table in 5.3.3 will assist in the interpretation of data.

Dimensions in millimetres

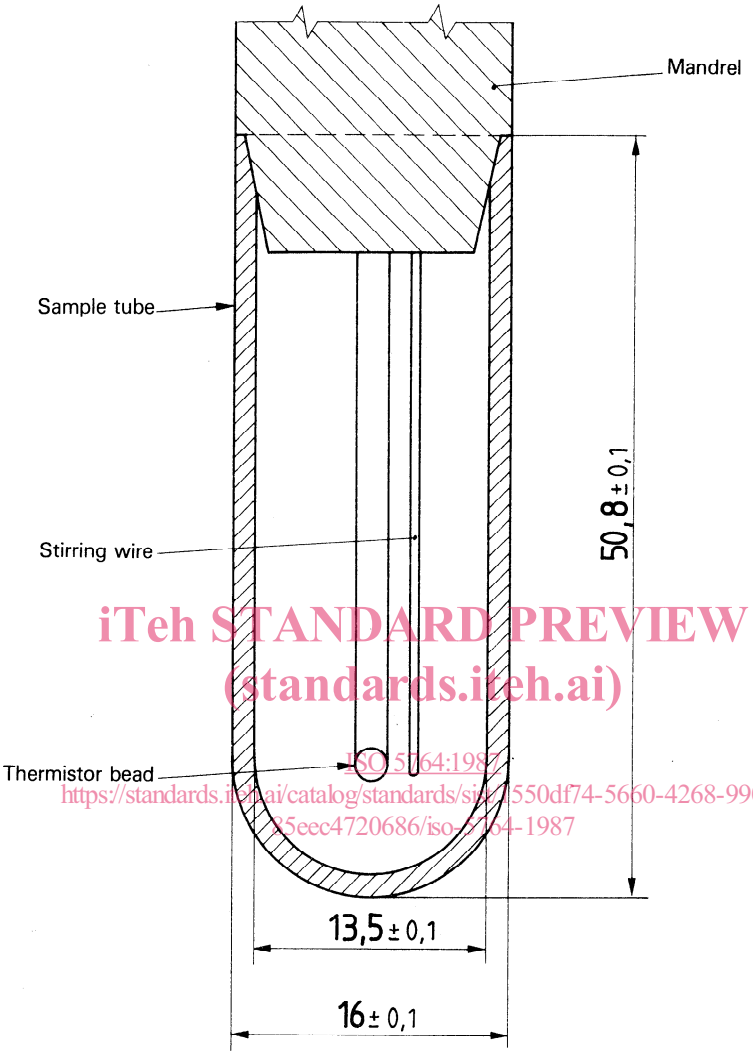

Figure — Detail of thermistor cryoscope (position of sample tube in relation to thermistor bead and stirring wire)

Bibliography

[1] Hortvet, J. *J. Ass. Off. Agric. Chem.* **5**, p. 470-484 (1922).

[2] *Bulletin of the International Dairy Federation* (1983), No. 154.

[3] *Bulletin of the International Dairy Federation* (1986), No. 207.

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