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Milk — Determination of total milk-clotting activity of bovine rennets

Lait — Détermination de l'activité totale de coagulation du lait dans la présure de bovins

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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 11815 | IDF 157 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 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 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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights.

ISO 11815 | IDF 157 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 Joint IDF-ISO Action Team on *Enzymes in cheese making*, of the Standing Committee on *Minor compounds and characterization of physical properties*, under the aegis of its project leader Mr A. van Boven (NL).

This edition of ISO 11815|IDF 157 cancels and replaces IDF 157A: 1997, which has been technically revised.

Introduction

Bovine rennets (calf and adult) contain, in various amounts, both chymosin and bovine pepsin as main milk-clotting enzymes. Each of these enzymes has its own characteristics as far as milk-clotting activity and cheese-making properties are concerned. The most obvious difference between these enzymes is the stronger pH dependence of the milk-clotting activity of pepsin. For economic reasons, therefore, it is very important to know the total milk-clotting activity of a certain rennet type and to have that characterized relative to an internationally recognized reference standard with known composition and milk-clotting activity.

The method is commonly known as the relative milk-clotting activity test (acronym: REMCAT).

A qualitative determination of the six most common milk-clotting enzymes in a sample is performed according to IDF 110B. In the case of mixtures of milk-clotting enzymes other than bovine chymosin and pepsin, no correct determination of the total milk-clotting activity for the sample can be obtained.

Milk — Determination of total milk-clotting activity of bovine rennets

1 Scope

This International Standard describes a method for the determination of the total milk-clotting activity of bovine rennet containing only chymosin and bovine pepsin as the active coagulating enzymes on a standard milk substrate at pH 6,5.

To produce accurate results with this method, test samples of unknown origin are to be checked for the absence of main milk-clotting enzymes of non-bovine origin by using an appropriate method (e.g. IDF 110B).

This International Standard can also be applied to determine the total milk-clotting activity of fermentation-produced chymosin.

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 648, *Laboratory glassware — One-mark pipettes*

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

IDF 110B:1997 *Calf rennet and adult bovine rennet — Determination of chymosin and bovine pepsin contents (Chromatographic method)*

3 Terms and definitions

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

3.1
total milk-clotting activity

amount of activity set relative to the first batch of international calf rennet reference standard powder and the first batch of adult bovine rennet reference standard powder, respectively

NOTE 1 For the first batch of both the calf and the adult bovine rennet reference standard powder, the activity was defined at 1 000 International Milk-Clotting Units per gram (IMCU/g). Future preparations of reference standards will be set relative to the previous reference standards.

NOTE 2 The total milk clotting activity of the calf and adult bovine rennet reference standard powders is approximately 1 000 IMCU/g, but their exact activity is stated on the certificate of analysis.

NOTE 3 The total proteolytic (milk-clotting) activity of these calf (or adult bovine) rennet reference standard powders is checked every second year by an alternative method, for example on a synthetic hexapeptide substrate by NIZO[1)] .

4 Principle

The time needed for a visible flocculation of a standard milk substrate prepared with a 0,5 g/l calcium chloride solution (pH ≈ 6,5) is determined. The clotting time of a rennet sample is compared under identical chemical and physical conditions to that of a reference standard with known milk-clotting activity and having the same enzyme composition as the sample, which was determined using IDF 110B.

5 Reagents

Use only reagents of recognized analytical grade, and distilled or demineralized water or water of equivalent purity.

5.1 Buffer solution, pH 5,5.

Add, using a pipette (6.1), 10,0 ml of 1 mol/l acetic acid (CH_3COOH) to 10,0 g of sodium acetate trihydrate ($CH_3COONa{\cdot}3H_2O$) and mix. Dilute with water to 1 000 ml. If necessary, adjust the pH to 5,5 with acetic acid or sodium acetate trihydrate.

5.2 Calcium chloride stock solution, $c(CaCl_2)$ = 500 g/l.

Calcium chloride solutions with the required accurate concentration of 500 g/l calcium chloride and the actual density stated are commercially available[2)]. Store the solution as specified by the manufacturer.

Prior to use, bring the calcium chloride stock solution to room temperature (18 °C to 22 °C). Check the concentration of the solution by titration with EDTA (ethylenediaminetetraacetic acid) every year.

5.3 Calcium chloride working solution, $c(CaCl_2)$ = 0,5 g/l.

Use the density of the stock solution (5.2) to calculate the mass needed to obtain a final amount of 0,5 g/l calcium chloride in the working solution.

The mass of the solution should be equivalent to the addition of 2,00 ml of the stock solution with the exact concentration required, $c(CaCl_2)$ = 500 g/l; in that case, the solution mass is ≈ 2,70 g.

Weigh, to the nearest 0,01 g, about 2,70 g of calcium chloride stock solution (5.2) of exactly known concentration at room temperature (18 °C to 22 °C) in a 2 000 ml one-mark volumetric flask. Dilute to the mark with water and mix. The calcium chloride solution shall be freshly prepared on the day of use.

Weighing of the calcium chloride stock solution (5.2) is recommended in order to prepare correctly the calcium chloride working solution as the viscous solution is difficult to pipette.

Alternatively, an intermediate calcium chloride solution of 50 g/l may be prepared and further diluted before use.

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1) Netherlands Institute for Dairy Research (NIZO), PO Box 20, 6710 BA Ede, The Netherlands. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of these products.

2) Ordering address: Chr. Hansen A/S, 1-27 Jernholmen, 2650 Hvidovre, Denmark (Fax: +45 36 86 77 76). This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of these products.

5.4 Low-heat, low-fat, spray-dried milk powders, of good renneting and bacteriological quality.

NOTE Low-heat, low-fat, spray-dried milk powders meeting the requirements are commercially available [2),3)].

5.5 Calf rennet reference standard powder [4)], in pouches of approx. 2,7 g powder, containing > 98 % chymosin and < 2 % bovine pepsin in terms of enzyme activity as determined according to IDF 110B.

The exact total milk-clotting activity is indicated on the certificate of analysis and should be about 1 000 IMCU/g.

The calf rennet reference standard powder is a primary reference standard; a secondary liquid standard may be made and used if it has been determined that the same result is obtained.

Store the calf rennet reference standard powder in the dark at −18 °C, protected against moisture. For short periods, for example during transport, it may be kept at ambient temperatures.

5.6 Adult bovine rennet reference standard powder [4)], in pouches of approx. 2,7 g powder, containing < 2 % chymosin and > 98 % bovine pepsin in terms of enzyme activity as determined according to IDF 110B.

The exact total milk-clotting activity is indicated on the certificate of analysis and should be about 1 000 IMCU/g.

The adult bovine rennet reference standard powder is a primary reference standard; a secondary liquid standard may be made and used if it has been determined that the same result is obtained.

Store the adult bovine reference standard powder in the dark at −18 °C, protected against moisture. For short periods, for example during transport, it may be kept at ambient temperatures.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Micropipette or any other pipette, capable of delivering 0,5 ml in less than 1 s with a repeatability of 0,2 % or better.

6.2 One-mark pipettes, in accordance with ISO 648, to deliver appropriate amounts.

Alternatively, a diluter (e.g. a Hamilton diluter) with the same high precision may be used for diluting the rennets. For measuring the substrate, a syringe or a dispenser delivering the appropriate amount with a repeatability of 0,4 % may also be used.

6.3 One-mark volumetric flasks, as specified in ISO 1042, of the required capacities.

6.4 Thermometer, calibrated, graduated between 20 °C and 45 °C, with a precision of ± 0,1 °C.

6.5 pH meter, capable of measuring the pH in 0,01 units.

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

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3) Institut national de la Recherche agronomique, France, which is dependent on the Station expérimentale laitière, BP 94, 39800 Poligny, France. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of these products.

4) AMAFE. Ordering address: Chr. Hansen A/S, 1-27 Jernholmen, 2650 Hvidovre, Denmark (Fax: +45 36 86 77 76). This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of these products.