TECHNICAL SPECIFICATION

ISO/TS 17837

IDF/RM 25

First edition
2008-03-15

Milk and milk products — Determination of nitrogen content and crude protein calculation — Kjeldahl method

Lait et produits laitiers — Détermination de la teneur en azote et calcul des protéines brutes — Méthode Kjeldahl

Reference numbers
ISO/TS 17837:2008(E)
IDF/RM 25:2008(E)

COPYRIGHT PROTECTED DOCUMENT

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org
Web www.fil-idf.org

Published in Switzerland

Contents

Page

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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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/TS 17837 was prepared by Technical Committee ISO/TC 34, *Food Products*, Subcommittee SC 5, *Milk and milk products*.

ISO/TS 17837|IDF/RM 25 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 at 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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a Standing Committee may decide to publish another type of normative document which is called by IDF: *Reviewed method*. Such a method represents an agreement between the members of a Standing Committee and is accepted for publication if it is approved by at least 50 % of the committee members casting a vote. A *Reviewed method* is equal to an ISO/PAS or ISO/TS and will, therefore, also be published jointly under ISO conditions.

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/TS 17837|IDF/RM 25 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 ISO-IDF Action Team on *Nitrogen compounds* of the Standing committee on *Main components in milk* under the aegis of its project leader, Mr. J. Romero (US).

This edition of ISO/TS 17837|IDF/RM 25 cancels and replaces IDF 25:1964, which has undergone minor editorial and technical revisions.

Milk and milk products — Determination of nitrogen content and crude protein calculation — Kjeldahl method

WARNING — Performance of the method specified in this Technical Specification may involve the use of hazardous materials, operations, and equipment. This Technical Specification does not purport to address all the safety risks associated with such performance. It is the responsibility of the user to establish appropriate safety and health practices and determine the applicability of local regulatory limitations prior to performance of the method.

1 Scope

This Technical Specification specifies a method for the determination of the nitrogen content and crude protein content by calculation in milk and milk products by using the Kjeldahl principle, both traditional and block digestion methods.

The methods specified are applicable to whole and skimmed liquid bovine, caprine and ovine milk, and hard, semi-hard and processed cheese.

NOTE Inaccurate crude protein results are obtained if non-dairy sources of nitrogen are present in the specified milk or milk products.

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 385, *Laboratory glassware — Burettes*

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

ISO 4788, *Laboratory glassware — Graduated measuring cylinders*

3 Terms and definitions

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

3.1
nitrogen content
mass fraction of nitrogen determined by the procedure specified in this Technical Specification

NOTE The nitrogen mass fraction is expressed as a percentage.

3.2
crude protein content
mass fraction of crude protein calculated as specified by this Technical Specification

NOTE The crude protein mass fraction is expressed as a percentage.

4 Principle

A test portion is digested with a mixture of concentrated sulfuric acid and potassium sulfate. Copper(II) sulfate is used as a catalyst to thereby convert organic nitrogen present to ammonium sulfate. The function of the potassium sulfate is to elevate the boiling point of the sulfuric acid and to provide a stronger oxidizing mixture for digestion. Excess sodium hydroxide is added to the cooled digest to liberate ammonia. The liberated ammonia is steam distilled into excess boric acid solution and titrated against a hydrochloric acid standard volumetric solution. The nitrogen content is calculated from the amount of ammonia produced and the crude protein content from the nitrogen content obtained.

5 Reagents

Unless otherwise specified, use only reagents of recognized analytical grade, and only distilled or demineralized water or water of equivalent purity.

5.1 Potassium sulfate (K_2SO_4), nitrogen free.

5.2 Copper(II) sulfate pentahydrate solution, $\rho(CuSO_4 \cdot 5H_2O)$ = 5,0 g/100 ml.

Dissolve 5,0 g of copper(II) sulfate pentahydrate in water in a 100 ml one-mark volumetric flask (6.8). Dilute to the mark with water and mix.

5.3 Sulfuric acid (H_2SO_4), with a mass fraction of between 95 % and 98 %, nitrogen free [$\rho_{20}(H_2SO_4) \approx 1,84$ g/ml].

5.4 Sodium hydroxide aqueous solution, nitrogen free, containing 50 g of sodium hydroxide (NaOH) per 100 g (mass fraction of sodium hydroxide, w_{NaOH} = 50 %).

If plugging of the flow system in an automatic distillation unit is a problem, use a solution with w_{NaOH} = 40 %.

5.5 Indicator solution.

5.5.1 Dissolve 0,1 g of methyl red in 95 % (volume fraction) ethanol in a 50 ml one-mark volumetric flask (6.8). Dilute to the 50 ml mark with similar ethanol and mix.

5.5.2 Dissolve 0,5 g of bromocresol green in 95 % (volume fraction) ethanol in a 250 ml one-mark volumetric flask (6.8). Dilute to the mark with similar ethanol and mix.

5.5.3 Mix one volume of the methyl red solution (5.5.1) with five volumes of the bromocresol green solution (5.5.2) or combine and mix all of both solutions.

5.6 Boric acid solution, $\rho(H_3BO_3)$ = 40,0 g/l.

Dissolve 40,0 g of boric acid (H_3BO_3) in 1 l hot water in a 1 000 ml one-mark volumetric flask (6.8). Allow the flask and its contents to cool to 20 °C. Make up to the mark with water, add 3 ml of indicator solution (5.5.3) and mix.

Store the solution, which is light orange in colour, in a borosilicate glass bottle. Protect the solution from light and sources of ammonia during storage.

NOTE If using electronic pH end-point titration, the addition of the indicator solution (5.5.3) to the boric acid solution can be omitted. On the other hand, the change in colour can also be used as a check on proper titration procedures.

5.7 Hydrochloric acid standard solution, $c(HCl) = (0,1 \pm 0,000\,5)$ mol/l.

The purchase of pre-standardized hydrochloric acid standard solution from a reputable manufacturer is recommended.

Using pre-standardized solutions avoids introduction of systematic errors when diluting a concentrated stock hydrochloric acid solution and then determining the molarity of the acid, a process which can give rise to poor reproducibility performance of the method. It also avoids the use of a standard solution for titration having a higher concentration than the mentioned upper limit (0,1 ± 0,000 5 mol/l), as that reduces the total titration volume per sample. In the latter case, the uncertainty in readability of the burette becomes a larger percentage of the value, which has a negative impact on the repeatability and reproducibility performance of the method. The same issues and additional sources of error arise when another acid (e.g. sulfuric acid) is substituted for hydrochloric acid. Such substitutions are therefore not recommended.

5.8 Ammonium sulfate [$(NH_4)_2SO_4$], with a minimum mass fraction assay of 99,9 % on dried material.

Immediately before use, dry the ammonium sulfate at 102 °C ± 2 °C for 2 h. Cool to room temperature in a desiccator.

5.9 Tryptophan ($C_{11}H_{12}N_2O_2$) or **lysine hydrochloride** ($C_6H_{14}N_2O_2 \cdot HCl$), with a minimum mass fraction assay of 99 %. When stored in a desiccator, it is not necessary to dry these reagents in an oven before use.

5.10 Sucrose ($C_{12}H_{22}O_{11}$), with a mass fraction of nitrogen of less than 0,002 %. Do not dry the sucrose in an oven before use.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Kjeldahl flasks, of capacity 500 ml or 800 ml.

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

6.3 Burette or automatic pipette, capable of delivering portions of 1,0 ml of copper(II) sulfate solution (5.2).

6.4 Graduated measuring cylinders, of capacities 50 ml, 100 ml and 500 ml, complying with the requirements of ISO 4788, class A.

6.5 Conical flasks, of capacity 500 ml, graduated at every 200 ml.

6.6 Burette, of capacity 50 ml, graduated at least at every 0,1 ml, complying with the requirements of ISO 385, class A. Alternatively, an automatic burette can be used fulfilling the same requirements.

6.7 Grinding device.

6.8 One-mark volumetric flasks, of capacities 50 ml, 100 ml, 250 ml, and 1 000 ml, complying with the requirements of ISO 1042, class A.

6.9 Boiling aids, e.g. calcined pumice, zinc dust, hard pieces of porcelain or high-purity amphoteric alundum (i.e. carborundum) granules, plain, mesh size 10. Do not reuse the aids.

NOTE Glass beads of approximately 5 mm diameter can also be used, but they may not promote boiling as efficiently as the alundum granules. More foaming problems may be encountered during digestion with glass beads.

6.10 Digestion apparatus, to hold the Kjeldahl flasks (6.1) in an inclined position (approximately 45°), with electric heaters or gas burners that do not heat the flasks above the level of their contents, and with a fume extraction system.

The heater source should be adjustable to control the maximum heater setting to be used during digestion. Preheat the heat source at the heater setting for evaluation. In the case of a gas heater, the preheated period shall be 10 min and for an electric heater, it shall be 30 min. For each of the heaters, determine the heater

setting that brings 250 ml of water including 5 to 10 boiling aids with an initial temperature of 25 °C to its boiling point in 5 min to 6 min. This is the maximum heater setting to be used during digestion.

6.11 Distillation apparatus (traditional method), made of borosilicate glass or other suitable material to which can be fitted a Kjeldahl flask (6.1) consisting of an efficient splash-head connected to an efficient condenser with straight inner tube and an outlet tube attached to its lower end. The connecting tubing and stopper(s) shall be close-fitting and preferably made of neoprene.

NOTE The distillation apparatus mentioned above may be replaced by the complete Parnas-Wagner distillation configuration (see Reference [4]) or other suitable equipment.

6.12 Digestion block, aluminium alloy block or equivalent block, fitted with an adjustable temperature control and device for measuring block temperature.

6.13 Digestion tubes, of capacity 250 ml, suitable for use with the digestion block (6.12).

6.14 Exhaust manifold, suitable for use with the digestion tubes (6.13).

6.15 Centrifugal scrubber apparatus or **filter pump** or **aspirator**, constructed of acid resistant material, for use with mains water supply.

6.16 Distillation unit (block digesting method), capable of steam distilling, manual or semi-automatic, suitable for accepting digestion tubes (6.13) and conical flasks (6.5).

6.17 Automatic titrator, provided with a pH-meter.

The pH-meter shall be calibrated properly in the range of pH 4 to pH 7 following normal laboratory pH-calibration procedures.

6.18 Spatula or suitable transfer device.

6.19 Filter paper, nitrogen-free, of dimensions and porosity suitable to hold the cheese test portion.

6.20 Water bath, capable of maintaining a temperature of between 38 °C and 40 °C.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have 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|IDF 50[1].

8 Preparation of the test sample

8.1 Cheese

Remove the rind, smear or mouldy surface layer of the cheese, in such a way as to provide a test sample representative of the cheese as it is usually consumed.

Grind (6.7) the representative test sample thus obtained. Quickly mix the whole mass and preferably grind the mass again quickly. Analyse the test sample as soon as possible after grinding.