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Milk and milk products — Determination of nitrogen content —

Part 1: Kjeldahl principle and crude protein calculation

Lait et produits laitiers — Détermination de la teneur en azote — Partie 1: Méthode Kjeldahl et calcul de la teneur en protéines brutes

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Contents

Page

Forewords

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2. www.iso.org/directives

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 34, *Food and food products*, Subcommittee SC 5, *Milk and milk products* and the International Dairy Federation (IDF) and is being published jointly by ISO and IDF.

This second edition of ISO 8968-1|IDF 20-1 cancels and replaces the first edition of ISO 8968-1|IDF 20-1:2001, ISO 8968-2|IDF 20-2:2001, ISO 5549:1978/IDF 92:1979 and ISO/TS 17837|IDF/RM 25:2008 which have been technically revised.

The International Dairy Federation (IDF) 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 Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of IDF National Committees casting a vote.

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ISO 8968-1|IDF 20-1 was prepared by the International Dairy Federation and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by ISO and IDF.

The work was carried out by the IDF-ISO Project Group on Nitrogen, of the Standing Committee on *Analytical Methods for Composition (SCAMC)*, under the aegis of its project leaders: Mr. R. Johnson (NZ), Mr. J. Romero (US), Dr. Barbano (US), Dr. Orlandini (IT), and Mr. Psathas (CY).

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Milk and milk products — Determination of nitrogen content —

Part 1:
Kjeldahl principle and crude protein calculation

WARNING — The use of this International Standard might involve the use of hazardous materials, operations, and equipment. This International Standard does not purport to address all the safety risks associated with its use. It is the responsibility of the user of this International Standard to establish appropriate safety and health practices and determine the applicability of local regulatory limitations prior to use.

1 Scope

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

The methods are applicable to:

— liquid cow's (whole, partially skimmed or skimmed milk), goat's and sheep's whole milk;

— hard, semi-hard and processed cheese;

— dried milk and dried milk products (including milk-based infant formulae, milk protein concentrate, whey protein concentrate, casein and caseinate).

The methods are not applicable to samples containing ammonium caseinate.

NOTE Inaccurate crude protein results will be obtained if non-milk sources of nitrogen are present in the products specified in this International Standard.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable to its application. 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 8655-3, *Piston-operated volumetric apparatus — Part 3: Piston burettes*

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 specified procedure

Note 1 to entry: It is expressed as a percentage.

3.2
crude protein content
mass fraction of crude protein calculated as specified

Note 1 to entry: It is expressed as a percentage.

4 Principle

A test portion is digested with a mixture of concentrated sulfuric acid and potassium sulfate. Using copper sulfate (II) as a catalyst to thereby convert any 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 the excess boric acid solution and titration with hydrochloric acid standard volumetric solution is carried out. The nitrogen content is calculated from the amount of ammonia produced.

5 Reagents

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

NOTE The solutions specified in this procedure might be different than those required for the operation of automated titrators. An effort is made to address those, but it is the responsibility of the operator to follow the directions of the equipment manufacturer.

5.1 Potassium sulfate (K_2SO_4), nitrogen free.

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

Dissolve 5,0 g of copper(II) sulfate pentahydrate in water in a 100 ml one-mark volumetric flask. 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 (approximately ρ_{20} = 1,84 g/ml).

5.4 Sodium hydroxide (NaOH) solution, nitrogen-free, containing 50 g of sodium hydroxide per 100 g.

With automated distillation systems, other mass fractions of sodium hydroxide may be used, provided an excess of sodium hydroxide is dispensed to the distillation mixture; for example, a mass fraction of 40 % sodium hydroxide solution may be used instead of a mass fraction of 50 %, where plugging of the automated flow system is a problem. The total volume of such sodium hydroxide solution should be considered in order to maintain the suitable distillation volumes.

5.5 Indicator solution

Dissolve 0,1 g of methyl red in 95 % (volume fraction) ethanol in a 50 ml one-mark volumetric flask (6.16). Dilute to 50 ml with ethanol and mix. Dissolve 0,5 g of bromocresol green in 95 % (volume fraction) ethanol in a 250 ml one-mark volumetric flask (6.16). Dilute to 250 ml with ethanol and mix. Mix one part of the methyl red solution with five parts of the bromocresol green solution or combine and mix all of both solutions.

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

Dissolve 40,0 g of boric acid (H_3BO_3) in 1 l of hot water in a 1 000 ml one-mark volumetric flask (6.16). Allow the flask and its contents to cool to 20 °C. Adjust to the mark with water, add 3 ml of the indicator

solution (5.5) and mix. Store the solution, which will be light orange in colour, in a borosilicate glass bottle. Protect the solution from light and sources of ammonia fume during storage.

With automated distillation systems, other boric acid concentrations may be used after validating accordingly.

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

5.7 Hydrochloric acid standard volumetric solution, c(HCl) = (0,1 ± 0,0005) mol/l.

It is recommended to purchase this material prestandardized by the manufacturer, which meets, or exceeds, these specifications. Often, the systematic errors (which can be avoided) introduced by an analyst diluting a concentrated stock acid and then determining the molarity of the acid, cause poor reproducibility performance of the method in this part. The analyst should not use a solution for titration that has a higher concentration than 0,1 mol/l, because this will reduce the total titration volume per sample and the uncertainty in readability of the burette will become a larger percentage of the value. This will have a negative impact on the method repeatability and reproducibility performance.

If sulfuric acid is substituted for hydrochloric acid, the solution should have a concentration of 0,05 ± 0,000 3 mol/l.

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

Immediately before use, dry the ammonium sulfate at 102 °C ± 2 °C for not less than 2 h. Cool to room temperature in a desiccator.

5.9 Tryptophan ($C_{11}H_{12}N_2O_2$) or lysine hydrochloride ($C_6H_{15}ClN_2O_2$), minimum assay 99 % (mass fraction).

Do not dry these reagents in an oven before use.

5.10 Sucrose, with a mass fraction of nitrogen of not more 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 Water bath, capable of maintaining a water temperature between 38 °C and 40 °C.

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

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

6.4 Graduated measuring cylinders, of capacity 25 ml, 50 ml, 100 ml and 500 ml.

6.5 Conical flasks, of capacity 500 ml.

6.6 Automatic burette, of suitable capacity e.g. 20 ml, with resolution of at least 0,004 ml, complying with the requirements of ISO 8655-3. Alternatively, a burette, of capacity 50 ml, graduated at least at every 0,1 ml, complying with the requirements of ISO 385, class A may be used for the analysis of milk.

NOTE The manual burette does not have sufficient resolution to achieve the required number of significant figures for all other products.

6.7 Grinding device

6.8 Digestion flasks (Kjeldahl), of 500 ml or 800 ml capacities. Suitable to the digestion system to be used and to the specifications of the manufacturer of the digestion apparatus (6.10 or 6.11).

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

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

6.10 Digestion apparatus, to hold the digestion flasks (6.8) in an inclined position (approximately 45 °), with electric heaters or gas burners, which 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 preheating 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 digestion flask (6.8) 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 polychloroprene.

NOTE The distillation apparatus mentioned above can be replaced by the complete Parnas-Wagner[1)] distillation configuration or other suitable equipment.

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

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

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

6.15 Centrifugal scrubber apparatus or filter pump or aspirator (block digesting method), constructed of acid-resistant material, for use with mains water supply.

6.16 Volumetric flasks, one mark of 50 ml, 250 ml and 1 000 ml capacities.

6.17 Distillation unit (block digesting method), capable of steam distilling, manual or semi–automatic, suited to accept the 250 ml digestion tubes (6.13) and the 500 ml conical flasks (6.5).

1) Parnas-Wagner is an example of glassware configuration utilized for Kjeldahl distillation available commercially. This information is given for the convenience of users and does not constitute an endorsement by either ISO or IDF of this product.

6.18 Automatic titrator provided with a pH-meter.

The pH-meter should be calibrated properly in the range of pH 4 to pH 7 following normal laboratory pH-calibration procedures. The automatic titrator burette shall comply with the requirements of 6.6.

6.19 Spatula or suitable transfer device.

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

6.21 Illuminated magnetic stirrer plate.

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.

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

8 Preparation of test sample

8.1 Whole, partially skimmed or skimmed liquid milk

Place the test sample in the water bath (6.1) set at 38 °C to 40 °C. Mix gently by inversion without causing frothing or churning. Once the sample is mixed thoroughly, cool to room temperature.

Proceed as indicated in 9.1 or 9.2.

8.2 Hard, semi-hard and processed 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 the test sample by means of an appropriate device (6.7). Quickly mix the whole mass and, preferably, grind the mass again quickly. Analyse the test sample as soon as possible after grinding.

Using the spatula (6.19), weigh the required amount (Table A.1) of either prepared ground cheese onto a pre-folded, tared filter paper (6.20). Enclose cheese in filter paper and drop the filter paper containing the cheese into the bottom of a digestion flask (6.8) or digestion tube (6.13) as indicated in 9.1.1 or 9.2.1.

NOTE Use of a filter paper can promote foam formation in block digestion systems. To avoid this, when using the block digestion method (9.2), the filter paper can be omitted by weighing the sample into a suitable vessel, weighing the cheese and vessel, transferring the cheese to the digestion vessel, reweighing the empty vessel and determining the sample mass by subtracting the mass of the empty vessel from the mass of the cheese and vessel.

8.3 Dried milk and dried milk products

Let the test sample reach a temperature of between 20 °C and 25 °C before transferring to a container of internal volume approximately twice the volume of the test sample. Close the container immediately to avoid changing the moisture content of the sample. Thoroughly mix the sample by repeatedly rotating and inverting the container.

Proceed as indicated in 9.1 or 9.2.