
**Meat and meat products —
Determination of nitrogen content —
Reference method**

*Viandes et produits à base de viande — Détermination de la teneur en
azote — 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.

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 document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 6, *Meat, poultry, fish, eggs and their products*.

This second edition cancels and replaces the first edition (ISO 937:1978), which has been technically revised.

The main changes are as follows:

- the automatic Kjeldahl method has been added;
- the order of the clauses of the document has been rearranged.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Meat and meat products — Determination of nitrogen content — Reference method

1 Scope

This document specifies a reference method for the determination of the nitrogen content of meat and meat products by the Kjeldahl principle.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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*

3 Terms and definitions

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

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

nitrogen content

quantity of nitrogen corresponding to the ammonia produced

Note 1 to entry: As determined using the conditions specified in this document.

4 Principle

Digestion of a test portion with concentrated sulfuric acid, using copper(II) sulfate as a catalyst, to convert organic nitrogen to ammonium ions; alkalization, distillation of the liberated ammonia into an excess of boric acid solution, titration with hydrochloric acid or sulfuric acid to determine the ammonia bound by the boric acid, and calculation of the nitrogen content of the sample from the amount of ammonia produced.

5 Sampling

Sampling is not part of the method specified in this document. A recommended sampling method is given in CAC/GL 50-2004.

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

Start from a representative sample of at least 200 g.

Store the sample in such a way that deterioration and change in composition are prevented. Preservatives, if any, should not contain nitrogen compounds in measurable amounts.

6 Preparation of test sample

Homogenize the laboratory sample with the appropriate equipment (8.1). Take care that the temperature of the sample material does not rise above 25 °C. If a mincer is used, pass the sample at least twice through the equipment.

Fill a suitable airtight container with the prepared test sample, close the container and store in such a way that deterioration and change in composition are prevented. Analyse the test sample as soon as practicable, but always within 24 h after homogenization.

7 Reagents and materials

All reagents shall be of recognized analytical quality. The water used shall be distilled water or water of at least equivalent purity.

7.1 Copper(II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$).

7.2 Potassium sulfate (K_2SO_4), anhydrous.

7.3 Sulfuric acid (H_2SO_4), ρ_{20} 1,84 g/ml.

7.4 Ethanol ($\text{C}_2\text{H}_5\text{OH}$), 95 % (volume fraction).

7.5 Ammonium sulfate [$(\text{NH}_4)_2\text{SO}_4$], minimum purity 99,9 % (mass fraction) on dried material.

Immediately before use, dry the ammonium sulfate at $104^\circ\text{C} \pm 4^\circ\text{C}$ for at least 2 h.

Allow it to cool at ambient temperature in a desiccator.

7.6 Tryptophan ($\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_2$) or lysine hydrochloride ($\text{C}_6\text{H}_{15}\text{ClN}_2\text{O}_2$) or acetanilide ($\text{C}_8\text{H}_9\text{NO}$), minimum purity 99,9 % (mass fraction).

These reagents should be kept away from humidity.

WARNING — Do not dry these reagents in an oven before use.

7.7 Sucrose, with nitrogen content less than a mass fraction of 0,002 %.

WARNING — Do not dry sucrose in an oven before use.

7.8 Sodium hydroxide (NaOH) solution, carbonate-free, containing approximately 33 g of sodium hydroxide per 100 g of solution.

Dissolve 500 g of sodium hydroxide in 1 000 ml of water.

7.9 Boric acid solution, $c(\text{H}_3\text{BO}_3) = 40,0$ g/l.

Dissolve 40 g of boric acid in water and dilute to 1 000 ml.

7.10 Standard hydrochloric acid, $c(\text{HCl}) = (0,1 \pm 0,000\ 5)$ mol/l or **sulfuric acid**, $c(\text{H}_2\text{SO}_4) = (0,05 \pm 0,000\ 3)$ mol/l, the normality being known to four decimal places.

7.11 Indicator solution, either [7.11.1](#) or [7.11.2](#).

7.11.1 Indicator solution A: mixed indicator (methyl red-methylene blue), prepared by dissolving 2 g of methyl red and 1 g of methylene blue in 1 000 ml of ethanol ([7.4](#)).

The colour change of this indicator solution occurs at a pH of 5,4.

7.11.2 Indicator solution B: mixed indicator (methyl red-bromocresol green), prepared by dissolving 1 g of methyl red and 5 g of bromocresol green in 1 000 ml of ethanol ([7.4](#)).

The colour change of this indicator solution occurs at a pH of 5,2.

Store the indicator solution in a brown bottle in a dark and cool place.

7.12 Boiling regulators, either [7.12.1](#) or [7.12.2](#).

7.12.1 For the digestion: glass beads, silicon carbide or splinters of hard porcelain.

7.12.2 For the distillation: silicon carbide or freshly ignited pieces of pumice stone.

8 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used.

8.1 Mechanical or electrical equipment capable of homogenizing the laboratory sample, including a high-speed rotational cutter or a mincer fitted with a plate with holes not exceeding 4,0 mm in diameter.

8.2 Greaseproof paper or filter paper, nitrogen-free, of dimensions porosity suitable to hold the test portion.

8.3 Burette, 50 ml, conforming to ISO 385, Class A.

8.4 Kjeldahl flask or digestion tube, of not more than 800 ml capacity, suitable to the digestion apparatus.

8.5 Digestion apparatus, to hold the Kjeldahl flask or digestion tube, with electric heaters or gas burners, fitted with an adjustable temperature control and device for measuring temperature.

8.6 Traditional Kjeldahl apparatus ([8.6.1](#) and [8.6.2](#)), or an **automatic Kjeldahl meter**, fitted with a distillation system, with or without a titration system.

8.6.1 Steam distillation apparatus or, alternatively, **ordinary distillation apparatus**.

8.6.2 Heating device, to provide heat source for distillation, fitted with an adjustable temperature control.

8.7 Analytical balance, capable of weighing to the nearest 0,001 g.

9 Procedure

9.1 Test portion

Place a few boiling regulators (7.12.1) into the Kjeldahl flask or digestion tube (8.4), then add about 0,5 g of the copper(II) sulfate (7.1) and 15 g of the anhydrous potassium sulfate (7.2).

Weigh, to the nearest 0,001 g, about 2 g of the test sample on a piece of greaseproof paper or filter paper (8.2).

Transfer the greaseproof paper or filter paper and the test portion to the Kjeldahl flask or digestion tube.

NOTE Samples with a high fat content can produce too much foam or spray during the digestion process, which will easily spill out of the Kjeldahl flask or digestion tube and cause inaccurate detection. If this phenomenon occurs during digestion, a smaller test portion or an antifoaming agent can be used to control the situation.

9.2 Determination

9.2.1 Traditional Kjeldahl apparatus

Add 25 ml of the sulfuric acid (7.3) to the Kjeldahl flask. Mix by gently swirling the liquid. If desired, a pear-shaped glass bulb may be inserted into the neck of the flask with its tapering end downwards.

Place the flask in an inclined position (at an angle of about 40° from the vertical position) on the digestion apparatus (8.5). At first, heat the flask gently until foaming has ceased and the contents have become completely liquefied. Then digest by boiling vigorously, occasionally rotating the flask, until the liquid has become completely clear and of a light blue-green colour. Keep the liquid boiling for another 90 min.

The total digestion time should not be less than 2 h. Take care that no condensed liquid runs down the outside of the flask. Prevent the escape of too much sulfuric acid caused by overheating during the digestion, as this is likely to result in a loss of nitrogen.

Cool to about 40 °C and cautiously add about 50 ml of water. Mix and allow to cool.

Pour into a conical flask, of capacity about 500 ml, 50 ml of boric acid solution (7.9) from a measuring cylinder. Add four drops of the indicator A (7.11.1) or B (7.11.2), mix and place the flask under the condenser of the distillation apparatus so that the outlet of the adapter dips into the liquid.

Treat the contents of Kjeldahl flask in one of the following ways:

- a) In the case of steam distillation: Transfer the contents of Kjeldahl flask to the distillation apparatus and rinse the flask with about 50 ml of water. Add 100 ml of the sodium hydroxide solution (7.8) by means of a measuring cylinder, pouring carefully along the inclined neck of the flask so that the two layers in the flask do not mix. Immediately attach the flask to the splash-head of the distillation apparatus. Heat the alkaline liquid by passing steam through it until boiling and keep it so for 20 min. At first heat gently to minimize foaming. The collected volume of distillate should be at least 150 ml.
- b) In the case of ordinary distillation: Cautiously dilute the contents of the Kjeldahl flask with about 300 ml of water and swirl. If desired, transfer to a 1 l flask. After about 15 min, add 100 ml of the sodium hydroxide solution (7.8) by means of a measuring cylinder, pouring carefully along the inclined neck of the flask so that the two layers in the flask do not mix. Immediately attach the flask to the splash-head of the distillation apparatus.

Distil at least 150 ml of liquid, even if the mixture bumps irregularly. Continue the distillation until the mixture starts bumping or until 250 ml of distillate has been collected. Make sure that the distillate is cooled effectively, and prevent the boric acid solution from becoming warm.

In either case, lower the conical flask just before terminating the distillation, so that the outlet of the adapter is above the liquid level. Rinse the outlet of the adapter above the liquid (internally and externally) with a little water. Verify the completion of the ammonia distillation with red litmus paper, wetted with distilled water; its colour should not be affected by the liquid dripping from the condenser. Stop heating. If the distillation is found to be incomplete, carry out a new determination, carefully following the instructions.

Titrate the contents of the conical flask with the hydrochloric acid solution or sulfuric acid (7.10). The end point is reached when the colour changes from green to violet (indicator A) or green to pink (indicator B). Record the volume of acid solution required, estimated to the nearest 0,02 ml.

Carry out two determinations on test portions taken from the same test sample.

9.2.2 Automatic Kjeldahl meter

Add 25 ml of the sulfuric acid (7.3) to the digestion tube. Place the digestion tube into the digestion apparatus and heat. When the temperature of the digestion apparatus reaches 420 °C, heat for another 1 h. The colour of the liquid in the digestion tube will become transparent green. Cool to about 40 °C and cautiously add about 50 ml of water. Mix and allow to cool.

Treat the contents of the digestion tube in one of the following ways:

- a) For an automatic Kjeldahl meter without an automatic titration system: Attach the digestion tube containing the mixed liquid to the distillation system. Place a conical flask containing 50 ml of boric acid solution (7.9) containing four drops of indicator A (7.11.1) or B (7.11.2) under the outlet of condenser. Set a programme to add 80 ml of the sodium hydroxide solution (7.8) into the digestion tube automatically and distil for around 5 min. The collected volume of distillate should be at least 150 ml. Remove the conical flask from the distillation system, then titrate the distillate with hydrochloric acid solution or sulfuric acid solution (7.10). The end point is reached when the colour changes from green to violet (indicator A) or green to pink (indicator B). Record the volume of hydrochloric acid solution or sulfuric acid solution required, estimated to the nearest 0,02 ml.
- b) For an automatic Kjeldahl meter with an automatic titration system: Place the digestion tube containing the mixed liquid to the sample rack. Set a programme to add 80 ml of the sodium hydroxide solution (7.8) into the digestion tube and 50 ml of the boric acid solution (7.9) containing four drops of indicator A (7.11.1) or B (7.11.2) into the titration vessel automatically. Then, to distil, titrate the distillate and record the data. Before the experiment, the titration end point should be set by type of indicator: for indicator A, the end point colour is violet, and for indicator B, the end point colour is pink. For different brands of Kjeldahl meter, refer to the manufacturer's instructions. The parameters of the programme can be adjusted to make it suitable for the instrument.

Carry out two determinations on test portions taken from the same test sample.

9.3 Blank test

Always perform a blank test (in duplicate) when fresh batches of reagents or freshly prepared solutions are used. It is advisable to carry out a blank test occasionally for reagents and solutions which have already been in use for some time.

Carry out this blank test using a piece of greaseproof paper or filter paper (8.2) in accordance with 9.1 and 9.2.

9.4 Recovery test

9.4.1 The accuracy of the procedure should be checked regularly by means of the recovery tests given in 9.4.2 or 9.4.4, carried out in accordance with 9.2.1 or 9.2.2.

9.4.2 Check that no loss of nitrogen occurs by using a test portion of 0,12 g of ammonium sulfate (7.5) and 0,85 g of sucrose (7.7).

NOTE The ammonium sulfate recovered check does not give information about the capability of the digestion conditions to release nitrogen, which is bound in the protein structures.

The percentage of nitrogen recovered shall be greater than 99 % for all position on the apparatus. For recoveries less than 99 %, the normality of the titrant may be higher than the stated value, or it is possible that nitrogen loss occurred in the digestion or distillation.

9.4.3 Check the efficiency of the digestion procedure by using the operating methods described above, replacing the test portion with 0,18 g of tryptophan or acetanilide or lysine hydrochloride (7.6) along with 0,67 g of sucrose.

At least a mass fraction of 98 % of the nitrogen shall be recovered. If the recovery is lower than 98 %, after having a mass fraction of 99 % to 100 % recovery on ammonium sulfate, the temperature or time of digestion is insufficient or there is undigested sample material on the inside of the Kjeldahl flask or digestion tube.

9.4.4 Lower results in either of the recovery tests (or higher than 100,0 % in 9.4.2) indicate failures in the procedure and/or inaccurate concentration of the standard volumetric hydrochloric acid solution or sulfuric acid (7.10).

10 Expression of results

The nitrogen content of the test portion, expressed as a percentage by mass, is equal to [Formula \(1\)](#):

$$0,014 \times c \times (V_1 - V_0) \times \frac{100}{m} \quad (1)$$

where

0,014 is the value, in grams, of the quantity of nitrogen equivalent to the use of 1 ml of a 0,5 mol/l sulfuric acid solution or 1 ml of a 1 mol/l hydrochloric acid solution;

c is the hydrochloric acid concentration, in mol/l, exactly used for titration; if sulfuric acid is substituted for hydrochloric acid, c is the exact molarity of the sulfuric acid multiplied by a factor of 2;

V_1 is the volume, in millilitres, of sulfuric acid or hydrochloric acid solution required for the determination;

V_0 is the volume, in millilitres, of sulfuric acid or hydrochloric acid solution required for the blank test;

m is the mass, in grams, of the test portion.

Take as the result the arithmetic mean of the results of the two determinations, if the requirement for precision (see [Clause 11](#)) is satisfied.

Report the result to the nearest 0,01 g of nitrogen per 100 g of sample.

11 Precision

The precision of the method has been established by an interlaboratory test, carried out in accordance with ISO 5725-1 and ISO 5725-2.

The absolute difference between two independent single test results, obtained using the same method on test material in the same laboratory by the same operator using the same equipment within a short interval of time, should not be greater than 0,10 g of nitrogen per 100 g of sample.

12 Notes on procedure

12.1 The determination should be carried out in a room free from ammonia vapour.

12.2 It is also possible to determine the nitrogen in an aliquot portion of the contents of the Kjeldahl flask. Under these conditions, suitable modifications to the apparatus and procedure will be required (quantities and concentrations of the reagents used, time of distillation, volume of distillate). These modifications should be shown in the test report.

12.3 Nitrogen originating from non-protein compounds will be included in the determination and will give inaccurate results for the protein content if the protein is calculated from the nitrogen content.

12.4 If, in addition to the result in terms of nitrogen, it is desired to express the results as protein, it is necessary to calculate protein content with the Kjeldahl factor. The Kjeldahl factor for meat and meat products is always 6,25.

13 Test report

The test report shall specify:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with reference to this document, i.e. ISO 937;
- all operating details not specified in this document, or regarded as optional, together with details of any incidents which can influence the test result(s);
- the test result obtained, or the two test results obtained if the repeatability has been checked;
- the date of the test.