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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 institutes (ISO member bodies). The work of developing International Standards is carried out through ISO technical committees. Every member body interested in a subject for which a technical committee has been set up 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.

International Standard ISO 937 was developed by Technical Committee ISO/TC 34, *Agricultural food products*.

It was submitted directly to the ISO Council, in accordance with clause 6.13.1 of the Directives for the technical work of ISO. It cancels and replaces ISO Recommendation R 937-1969, which had been approved by the member bodies of the following countries :

Australia	Hungary	Romania
Bulgaria	India	South Africa, Rep. of
Canada	Iran	Thailand
Colombia	Ireland	Turkey
Czechoslovakia	Israel	United Kingdom
Egypt, Arab Rep. of	Korea, Rep. of	U.S.S.R.
France	Norway	Yugoslavia
Germany, F.R.	Poland	
Greece	Portugal	

The member bodies of the following countries had expressed disapproval of the document on technical grounds :

Netherlands
New Zealand

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

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a reference method for the determination of the nitrogen content of meat and meat products.¹⁾

2 REFERENCE

ISO 3100, *Meat and meat products — Sampling*.

3 DEFINITION

nitrogen content of meat and meat products : The quantity of nitrogen corresponding to the ammonia produced and determined under the conditions specified below.

4 PRINCIPLE

Digestion of a test portion with concentrated sulphuric acid, using copper(II) sulphate as a catalyst, to convert organic nitrogen to ammonium ions; alkalisation, distillation of the liberated ammonia into an excess of boric acid solution, titration with hydrochloric 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 REAGENTS

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

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

5.2 Potassium sulphate (K_2SO_4), anhydrous.

5.3 Sulphuric acid, ρ_{20} 1,84 g/ml.

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

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

5.5 Boric acid solution.

Dissolve 40 g of boric acid (H_3BO_3) in water and dilute to 1 000 ml.

5.6 Hydrochloric acid, 0,1 N standard volumetric solution, the normality being known to four decimal places.

5.7 Indicator solution : mixed indicator (methyl red-methylene blue)²⁾, prepared by dissolving 2 g of methyl red and 1 g of methylene blue in 1 000 ml of 95 % (V/V) ethanol.

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

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

5.8 Boiling regulators.

5.8.1 For the digestion

Glass beads, silicon carbide or splinters of hard porcelain.

5.8.2 For the distillation

Silicon carbide or freshly ignited pieces of pumice stone.

6 APPARATUS

Usual laboratory apparatus not otherwise specified, and the following items :

6.1 Mechanical meat mincer, laboratory size, fitted with a plate with holes of diameter not exceeding 4 mm.

6.2 Greaseproof paper, pieces about 9 cm × 6 cm.

6.3 Burette, 50 ml, complying with ISO/R 385, class A.

6.4 Kjeldahl flask, of not more than 800 ml capacity, provided, if desired, with a pear-shaped glass bulb loosely fitting on top of the neck.

1) See also ISO 1871, *Agricultural food products — General directions for the determination of nitrogen by the Kjeldahl method*.

2) Sometimes known as *Tashiro* indicator.

6.5 Steam distillation apparatus or, alternatively, ordinary distillation apparatus.

6.6 Heating device, on which the Kjeldahl flask can be heated in an inclined position in such a way that the source of heat touches only that part of the flask wall which is below the liquid level. For gas heating, a suitable device is a plate of asbestos provided with a circular hole, so that only the lower part of the flask is exposed to the free flame.

6.7 Effective suction device, for the acid fumes evolved during the digestion.

6.8 Analytical balance.

7 SAMPLE

7.1 Proceed from a representative sample of at least 200 g. See ISO 3100.

7.2 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.

8 PROCEDURE

8.1 Preparation of the test sample

Homogenize the sample by passing it at least twice through the meat mincer (6.1) and mixing. Keep it in a completely filled, air-tight, closed container and store it in such a way that deterioration and change in composition are prevented. Analyse the sample as soon as possible after homogenization, but always within 24 h.

8.2 Test portion

Place a few boiling regulators (5.8) into the Kjeldahl flask (6.4), then add about 15 g of the anhydrous potassium sulphate (5.2) and 0,5 g of the copper(II) sulphate (5.1).

Weigh, to the nearest 0,001 g, about 2 g (or 1,5 g in the case of a sample rich in fat) of the test sample (8.1) on a piece of greaseproof paper (6.2).

Transfer the greaseproof paper and the test portion to the Kjeldahl flask.

8.3 Determination

Add 25 ml of the sulphuric acid (5.3) 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 heating device (6.6). 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 sulphuric 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 the boric acid solution (5.5) from a measuring cylinder, add 4 drops of the indicator solution (5.7), mix and place the flask under the condenser of the distillation apparatus (6.5) so that the outlet of the adapter dips into the liquid.

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

a) *In the case of steam distillation*

Transfer the contents of the Kjeldahl flask to the distillation apparatus and rinse the flask with about 50 ml of water. Add 100 ml of the sodium hydroxide solution (5.4) 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 litre flask. After about 15 min, add 100 ml of the sodium hydroxide solution (5.4) 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 (5.6). Record the volume of hydrochloric acid solution required, estimated to the nearest 0,02 ml.

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

8.4 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 according to 8.3, taking a piece of greaseproof paper (6.2) only.

9 EXPRESSION OF RESULTS

9.1 Method of calculation and formula

The nitrogen content of the sample, expressed as a percentage by mass, is equal to

$$0,001\,4 \times (V_1 - V_0) \times \frac{100}{m}$$

where

V_0 is the volume, in millilitres, of 0,1 N hydrochloric acid solution required for the blank test;

V_1 is the volume, in millilitres, of 0,1 N hydrochloric acid solution required for the determination;

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

NOTE — If the standard volumetric hydrochloric acid solution used is not of exactly the concentration indicated in 5.6, a suitable correction factor should be used in calculating the result.

Take as the result the arithmetic mean of the results of the two determinations, if the requirement for repeatability (see 9.2) is satisfied.

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

9.2 Repeatability

The difference between the results of two determinations carried out almost simultaneously or in rapid succession by the same analyst should not be greater than 0,10 g of nitrogen per 100 g of sample.

10 NOTES ON PROCEDURE

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

10.2 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.

10.3 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.

If, in addition to the result in terms of nitrogen, it is desired to express the results as protein, it is necessary to indicate the factor used.

11 TEST REPORT

The test report shall show the method used and the result obtained, as nitrogen; if the result in terms of protein is also required, the factor used shall also be given. It shall mention any operating conditions not specified in this International Standard, or regarded as optional (in particular, if the determination has been carried out on an aliquot portion — see 10.2), as well as any circumstances that may have influenced the result.

The report shall include all details required for the complete identification of the sample.

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