INTERNATIONAL STANDARD

ISO 16634-1

First edition
2008-11-01

Food products — Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content —

Part 1: Oilseeds and animal feeding stuffs

Produits alimentaires — Détermination de la teneur en azote total par combustion selon le principe Dumas et calcul de la teneur en protéines brutes —

Partie 1: Graines oléagineuses et aliments des animaux

Reference number
ISO 16634-1:2008(E)

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Published in Switzerland

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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 16634-1 was prepared by Technical Committee ISO/TC 34, *Food products*.

ISO 16634 consists of the following parts, under the general title *Food products — Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content*:

— *Part 1: Oilseeds and animal feeding stuffs*

A part 2 on cereals, pulses and milled cereal products is in preparation.

Introduction

For a long time the Kjeldahl method has been the most frequently used method for the determination of protein content of food products. However, in recent years, the Kjeldahl method has increasingly been replaced by the Dumas method, which is faster and does not use dangerous chemicals. Although the principles of the two methods are different, both measure the nitrogen content of the product. Nitrogen can be converted into protein content by using an appropriate factor. The value of this factor varies with the relative amounts of different proteins and their amino-acid composition in the given product.

Neither the Dumas nor the Kjeldahl method distinguishes between protein and non-protein nitrogen. In most cases, results obtained by the Dumas method are slightly higher than those of the Kjeldahl method. This is due to the fact that the Dumas method measures almost all of the non-protein nitrogen, whereas the Kjeldahl method measures only a part of it.

Taking into consideration that the calculated protein content of a product by both methods only approximates the true value, it is a matter of discretion which one is accepted. The most appropriate solution should be the use of a second factor for the elimination of the systematic error caused by the non-protein nitrogen content of the different products. However, this second factor has to be determined for each product, like existing factors, which show the ratio of the protein to the nitrogen content.

Food products — Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content —

Part 1: Oilseeds and animal feeding stuffs

1 Scope

This part of ISO 16634 specifies a method for the determination of the total nitrogen content and the calculation of crude protein content of oilseeds and animal feeding stuffs.

This method, like the Kjeldahl method, does not distinguish between protein nitrogen and non-protein nitrogen. For the calculation of protein content, various conversion factors are used (see Annex D).

This method is not applicable to milk and milk products, for which a method is specified in ISO 14891 | IDF 185[10].

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 664, *Oilseeds — Reduction of laboratory sample to test sample*

ISO 665, *Oilseeds — Determination of moisture and volatile matter content*

ISO 771, *Oilseed residues — Determination of moisture and volatile matter content*

ISO 6496, *Animal feeding stuffs — Determination of moisture and other volatile matter content*

ISO 6498, *Animal feeding stuffs — Preparation of test samples*

3 Terms and definitions

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

3.1
nitrogen content
mass fraction of the total nitrogen determined by the procedure specified in this part of ISO 16634

NOTE The mass fraction is expressed as a percentage.

3.2
crude protein content
nitrogen content (3.1) multiplied by a factor, usually 6,25

NOTE 1 A listing of other factors for possible use with various commodities is given in Annex D.

NOTE 2 The factors for calculation of crude protein content from the total content of nitrogen are derived from the Kjeldahl method which is the reference method for the determination of total nitrogen content. As the method uses the same factors as the Kjeldahl method, the use of these factors has to be verified due to the slight difference in results between the Kjeldahl and Dumas methods.

4 Principle

Samples are converted to gases by heating in a combustion tube which gasifies samples. Interfering components are removed from the resulting gas mixture. The nitrogen compounds in the gas mixture or a representative part of them are converted to molecular nitrogen, which is quantitatively determined by a thermal conductivity detector. The nitrogen content is calculated by a microprocessor.

5 Reagents

Use only reagents of recognized analytical grade, or reagents of equivalent purity as specified by instrument manufacturers. Except for the reference materials (5.12), all reagents shall be free from nitrogen.

5.1 Carrier gas(es): use one of 5.1.1 and 5.1.2.

5.1.1 Carbon dioxide, as pure as possible and of minimum volume fraction, $\varphi(CO_2) \geqslant 99{,}99$ %.

5.1.2 Helium, as pure as possible and of minimum volume fraction, $\varphi(He) \geqslant 99{,}99$ %.

5.2 Oxygen, as pure as possible and of minimum volume fraction, $\varphi(O_2) \geqslant 99{,}99$ %.

5.3 Sulfur dioxide and halogen absorbent, to eliminate any sulfur from the sample [e.g. lead chromate ($PbCrO_4$) or steel wool].

5.4 Copper oxide platinum catalyst (filling material for the post-combustion tube).

Platinum catalyst [5 % of Pt on alumina (Al_2O_3)] is blended with CuO at a ratio of 1:7 parts or 1:8 parts according to the manufacturer's recommendations.

To prevent separation as a result of the different bulk densities of the two materials, it is recommended not to prepare the mixture before filling the tube. It is advisable to pour the platinum catalyst and copper oxide simultaneously into the post-combustion tube using a suitable funnel.

5.5 Silver or copper wool.

This should be disaggregated before being inserted in the post-combustion or reduction tube.

5.6 Silica (quartz) or **glass wool** or **cotton wool**, as recommended by the instrument manufacturer.

5.7 Copper (wire, cuttings, turnings or **powder)**, or **tungsten** for the reduction tube.

The use of copper wires can improve the precision of analytical results for samples with low nitrogen contents (about 1 % mass fraction).

5.8 Diphosphorus pentoxide (P_2O_5) or **granulated magnesium perchlorate** [$Mg(ClO_4)_2$], or another suitable support material, to fill the drying tubes.

5.9 Hollow corundum spheres or **aluminium oxide pellets**, for the combustion tube.

5.10 Copper oxide (CuO), as filling material for the combustion tube.

5.11 Sodium hydroxide (NaOH), on a support material.

5.12 Aspartic acid ($C_4H_7NO_4$) or **ethylenediaminetetraacetic acid** ($C_{10}H_{16}N_2O_8$) or **glutamic acid** ($C_5H_9NO_4$) or **hippuric acid** ($C_9H_9NO_3$) **standard**, or other suitable reference materials with known, constant, certified nitrogen content.

Minimum recovery should be 99 % mass fraction.

5.13 Light petroleum, with boiling range between 30 °C and 60 °C, or **acetone** or **ethanol**.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Analytical balance, capable of weighing to the nearest 0,000 1 g.

6.2 Grinding device, appropriate to the nature of the sample.

6.3 Sieve, of nominal size of openings 800 µm or 1 mm, made of non-ferrous material.

6.4 Crucibles (e.g. made of stainless steel, quartz, ceramic or platinum) or **tin capsules** or **nitrogen-free filter paper for pressing pellets**, suitable for the Dumas apparatus used.

NOTE 1 Several commercial instruments are provided with an automatic sampler.

NOTE 2 Some solid samples (e.g. powders) can be pressed to form pellets.

6.5 Dumas apparatus[1], fitted with a furnace able to maintain a given temperature greater than or equal to 850 °C, with a thermal conductivity detector and suitable device for signal integration.

Suitable Dumas apparatus operates according to the general flowchart given in Annex A, although different arrangements and components may be used.

NOTE Schemes of three available instruments are shown as examples in Figures B.1, B.2, and B.3.

To avoid leaks, the sealing O-rings shall be slightly lubricated with high-vacuum grease prior to installation.

Experience has shown that it is important to clean all pieces of silica and glassware carefully, and to remove fingerprints from the tubes using a suitable solvent (e.g. acetone) before inserting them in the furnace.

1) Elementar Analysensysteme, Sumika Chemical Analysis Service, and LECO Instruments produce suitable equipment available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this equipment. Equivalent products may be used if they can be shown to lead to the same results.

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 part of ISO 16634. Recommended sampling methods are given in ISO 542[1] for oilseeds, in ISO 5500[3] for oilseed residues, and in ISO 6498 for animal feeding stuffs.

8 Preparation of test sample

The laboratory sample shall be prepared in such a way that a homogeneous test sample is obtained, which is representative of the oilseeds (see ISO 664) or animal feeding stuff (see ISO 6498).

Using a suitable grinding device (6.2), grind the laboratory sample. Generally, pass the ground material through a sieve (6.3) of nominal size of openings 800 µm for small sample sizes (under 300 mg), or a sieve of nominal size of openings 1 mm, for larger sample sizes (300 mg or more)[15]. Mills that produce particle sizes meeting the specifications given in Table 1 will give acceptable results.

Table 1 — Required particle size

Nominal size of sieve openings µm	Amount passing through sieve % mass fraction
710	100
500	95 to 100
200	85 or less

Grinding may result in moisture loss and therefore the moisture content of the ground sample should also be analysed when reporting nitrogen or protein values to dry matter or a constant moisture basis. Determination of the moisture shall be carried out according to ISO 665, ISO 771 or ISO 6496.

The grinder efficiency may be checked by replicate preparation of ground samples of a 2+1 mixture of corn and soya seeds. The expected coefficient of variation should be less than 2 % mass fraction.

9 Procedure

9.1 General

Carefully follow the manufacturer's instructions for instrument set-up, optimization, calibration and operation. Switch the instrument on and allow it to stabilize as defined in local procedures.

An instrument performance test should be made daily, using the reference material (5.12). The recovery of nitrogen should be $>$ 99,0 % mass fraction.

9.2 Test portion

Weigh, to the nearest 0,000 1 g, at least 0,1 g of the test sample into a crucible or tin capsule or nitrogen-free filter paper for pressing pellets (6.4). For samples low in protein ($<$ 1 % mass fraction), the amount of the test portion may be increased up to 3,5 g, depending on both the type of the Dumas equipment used and the nature of the test portion.

Depending on the type of equipment used, if the samples contain over 17 % mass fraction moisture, it may be necessary to dry them before analysis.

Lower masses may be necessary for very high protein content samples or where only very small amounts of sample are available. In the case of masses less than 0,1 g, a validation shall be performed.

9.3 Control of oxygen demand

Control oxygen demand, in particular the flow, according to the instructions of the material supplier.

Conduct as many blanks as necessary to stabilize the equipment, each using an equivalent mass of sucrose in place of the sample, with each set of nitrogen or protein determinations to mimic the test sample run. The sucrose blank provides the amount of nitrogen that is introduced by the atmospheric gases and is trapped within a powdered organic material source. Use the mean value of the atmospheric blank determinations as an error correction in the calculation of the nitrogen or protein determination of each test sample.

9.4 Calibration

Use pure compounds with known constant nitrogen content, e.g. aspartic acid (5.12), as standards for long-term instrument calibration. Analyse, in duplicate, three pure compounds, each with three different concentrations, chosen according to the measurement range of the actual samples.

To prepare a calibration curve, choose the compound and the amount used to ensure that an absolute amount of nitrogen in connection with the matrices to be analysed can be detected. For calibration, five standard samples (minimum) should be used, according to the scope of the analysed matrices.

Above 200 mg of nitrogen, the calibration curve is expected to be non-linear. In this non-linear section, several short segments may be used for calibration. To ensure the quality of calibration in this region, standard samples should be increased in steps of 1 mg to 5 mg of nitrogen.

Calibration may also be performed using aqueous standard solutions.

Check the calibration at least three times at the beginning of the series, and then every 15 to 25 samples, analysing either one of the replicate standards, or a sample of known value. The value obtained shall be less than 0,05 % mass fraction nitrogen of the expected value. Otherwise, analyse the samples again after checking instrument performance.

9.5 Determination

With the instrument under operating conditions, introduce the test portion according to the manufacturer's instructions.

During analysis, the following processes take place in the instrument (see Figure B.1, B.2 or B 3).

The test portion is quantitatively combusted under standardized conditions at a temperature of 850 °C minimum, depending on the instrument and the material being tested.

Volatile decomposition products (mainly molecular nitrogen, nitrogen oxides, carbon dioxide, and water vapour) are transported by the carrier gas (5.1) through the instrument.

Nitrogen oxides are reduced to molecular nitrogen and the excess of oxygen is bound to the copper or tungsten in the reduction column (5.7).

Water is removed by means of a condenser filled with magnesium perchlorate, diphosphorus pentoxide or other drying agents (5.8). Unless carbon dioxide is used as carrier gas (5.1.1), it is removed by being passed over a suitable absorbent, e.g. sodium hydroxide on a support material (5.11).

Interfering compounds (e.g. volatile halogen and sulfur compounds) are removed by absorbents (5.3) or contact materials [e.g. silver wool (5.5) or sodium hydroxide on a suitable support material (5.11)].