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Cereals, pulses, milled cereal products, oilseeds and animal feeding stuffs — Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content

Céréales, légumineuses, produits céréaliers de mouture, graines oléagineuses et aliments des animaux — Détermination de la teneur en azote total par combustion selon le principe Dumas et calcul de la teneur en protéines brutes

ICS 65.120; 67.060; 67.200

ISO/CEN PARALLEL ENQUIRY

The CEN Secretary-General has advised the ISO Secretary-General that this ISO/DIS covers a subject of interest to European standardization. **In accordance with the ISO-lead mode of collaboration as defined in the Vienna Agreement, consultation on this ISO/DIS has the same effect for CEN members as would a CEN enquiry on a draft European Standard.** Should this draft be accepted, a final draft, established on the basis of comments received, will be submitted to a parallel two-month FDIS vote in ISO and formal vote in CEN.

To expedite distribution, this document is circulated as received from the committee secretariat. ISO Central Secretariat work of editing and text composition will be undertaken at publication stage.

Pour accélérer la distribution, le présent document est distribué tel qu'il est parvenu du secrétariat du comité. Le travail de rédaction et de composition de texte sera effectué au Secrétariat central de l'ISO au stade de publication.

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Foreword

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

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 the 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 but 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 decision 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 must be determined for each product, similarly to the existing factors, which show the ratio of the protein and the nitrogen content.

Cereals, pulses, milled cereal products, oilseeds and animal feeding stuffs — Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content

1 Scope

This International Standard specifies a method for the determination of the total nitrogen content and the calculation of crude protein content of cereals, pulses, milled cereal products, oilseeds and animal feeding stuffs.

This method, similarly to 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 I IDF 185.

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 712, *Cereals and cereal products — Determination of moisture content — Routine reference method*

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*

ISO 6540, *Maize — Determination of moisture content (on milled grains and on whole grains)*

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 International Standard

NOTE The nitrogen content is expressed as a mass fraction in percent.

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

NOTE 1 The factor 5,7 is used for wheat, rye and their milled products.

NOTE 2 A listing of other factors for possible use with various commodities is given in Annex D.

NOTE 3 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 gasifies samples. All interfering components are removed from the resulting gas mixture. The nitrogen compounds of the gas mixture or a representative part of them are/is 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 the following.

5.1.1 Carbon dioxide (CO_2), as pure as possible and not less than 99,99 % (volume fraction).

5.1.2 Helium (He), as pure as possible and not less than 99,99 % (volume fraction).

5.2 Oxygen (O_2), as pure as possible and not less than 99,99 % (volume fraction).

5.3 SO_2 and halogens 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 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 not recommended 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), for the reduction tube.

Alternatively tungsten may be used as a catalyst.

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

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 **pellets of aluminium oxide**, 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 assay should be a mass fraction of 99 %.

5.13 Light petroleum, with boiling range between 30 °C and 60 °C.

Alternatively acetone or ethanol may be used.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Analytical balance, capable of weighing to the nearest 0,0001 g.

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

6.3 Sieve, of aperture size 0,80 mm or 1 mm made of iron-free material.

6.4 Crucibles (e.g. made of stainless steel, quartz, ceramic or platinum) or **tin capsules**, 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 then or equal to 850 °C, with a thermal conductivity detector and suitable device for signal integration.

Suitable types of Dumas apparatus available on the market operate 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 the Annex B (Figures B.1, B.2 and B.3).

To avoid leaks, the O-rings used for sealing 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.

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1) Dumas apparatus from the manufacturers Elementar Analysensysteme GmbH, Sumika Chemical Analysis Service, Ltd and LECO Instruments are examples of 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.

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. Recommended sampling methods are given in ISO 542 for oilseeds, in ISO 5500 for oilseed residues, in ISO 13690 for cereals, pulses and milled products and in ISO 6497 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 for the product ISO 664 or ISO 6498.

Using a suitable grinding device (6.2), grind the laboratory sample in order to obtain a relative standard deviation (*RSD*) of less 2 % on all products (over 10 successive runs). Generally, this condition is met when the ground samples pass through the sieve (6.3), aperture 0,80 mm, for small sample sizes (under 300 mg), or the sieve, aperture 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

Aperture size of sieve (µm)	Amount passing through sieve (%)
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 the relevant International Standards: ISO 665, ISO 712, ISO 771, ISO 6496 or ISO 6498.

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

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 reference material according to 5.12. The recovery of nitrogen should be > 99,0 %.

A method performance and applicability test shall be carried out for each type of sample material for each batch, using reference materials with certified nitrogen content.

9.2 Test portion

Weigh at least 0,1 g of the test sample to the nearest 0,0001 g, into a crucible or tin capsule (6.4). For samples low in protein (a mass fraction of < 1 %), 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.

If samples contain over 17 % moisture, dry them before analysis.

9.3 Control of oxygen demand

Some types of Dumas equipment require an estimate of the oxygen demand of the test portion. The calculated oxygen demand of some compounds used for calibration is given in Annex C. For instruments with a self-optimizing oxygen control, a residual oxygen content between 2 % and 8 % is required.

Conduct five atmospheric blank determinations, 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 longterm 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, the compound and the amount used should be chosen to ensure that an absolute amount of nitrogen between 4 mg and 200 mg can be detected. For calibration, use 10 to 20 (or more) standard samples to equally cover the mass range up to 200 mg of nitrogen. 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 assure 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.

Before starting a series of determinations, check instrument response by running at least three replicate standards of known content. When the response is constant and the values obtained correspond to the long-term calibration as established above, proceed with the determination of the daily calibration factor by analysing at least four replicate standard samples representing a nitrogen mass higher than the samples to be analysed.

Use this factor for calibration of the measurement series.

Full-range re-calibration is necessary if the daily calibration factor deviates from its expected value by more than 10 %, or if essential parts of the instrument that have a direct influence on calibration (e.g. thermal conductivity detector) have been replaced.

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 temperatures between 850 °C and 1 200 °C depending on the instrument and the material being tested.

Volatile decomposition products (mainly N_2, NO_x, CO_2, H_2O) are transported by the carrier gas (5.1) through the instrument.

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

Water is removed by means of a condenser filled by magnesium diphosphorus pentoxid 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 supporting 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)].

The nitrogen in the residual gas mixture consisting of nitrogen and carrier gas is passed through a thermal conductivity detector.

9.6 Detection and integration

For quantitative nitrogen determination the instrument uses a sensitive thermal conductivity cell that is optimized for the carrier gas employed and which may have automatic zero adjustment between the measurement of the test portions. After amplification and analog/digital conversion of the detector signal, data obtained are processed by peripheral microprocessor hardware.

10 Calculation and expression of results

10.1 Calculation

10.1.1 Nitrogen content

Results for the total nitrogen content, w_N, expressed as a mass fraction in percent, are usually available from instrument printouts.

10.1.2 Crude protein content

The crude protein content, w_p, expressed as a mass fraction in percent, is obtained by using the following equation:

$$w_p = w_N \cdot F$$

where

w_N is the numerical value of the nitrogen content, expressed as a mass fraction in percent of the sample at its natural moisture content,

F is the agreed ratio factor that is 5,7 for wheat, rye and their milled products, and 6,25 for other commodities (see also Annex D).

On request the crude protein content may be expressed as a mass fraction of the dry matter by using the following equation:

$$w_{pd} = \frac{100 w_p}{100 - w_m}$$

where

w_{pd} is the numerical value of the crude protein content, expressed as a mass fraction in percent of the dry matter,

w_m is the numerical value of the moisture content, expressed as a percentage by mass, determined according to ISO 665, ISO 712, ISO 771, ISO 6496 or ISO 6498.

10.2 Expression of results

Express the results to three significant figures (e.g. 9,53 % or 20,5 % or 35,4 %).

11 Precision

11.1 Interlaboratory tests

Details of interlaboratory tests on the precision of the method are summarized in Annex E.

The values derived for these interlaboratory tests may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than

a mass fraction of 0,1 % if the sample contains less than a mass fraction of 4 % nitrogen, and

2 % of the nitrogen content if the sample contains a mass fraction of 4 % or more nitrogen.

11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater

a mass fraction of 0,17 % if the sample contains less than a mass fraction of 4 % nitrogen, and

4 % of the nitrogen content if the sample contains a mass fraction of 4 % or more nitrogen.

12 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incident which may have influenced the test result(s);

e) the test result(s) obtained and the conversion factor used;