



BSI Standards Publication

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

Part 2: Cereals, pulses and milled cereal
products (ISO 16634-2:2016)

National foreword

This British Standard is the UK implementation of EN ISO 16634-2:2016. It supersedes DD CEN ISO/TS 16634-2:2009 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/4, Cereals and pulses.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Produits alimentaires - Détermination de la teneur en azote total par combustion selon le principe Dumas et calcul de la teneur en protéines brutes - Partie 2: Céréales, légumineuses et produits céréaliers de mouture (ISO 16634-2:2016)

Lebensmittelerzeugnisse - Bestimmung des Gehaltes an Gesamtstickstoff mit dem Verbrennungsverfahren nach Dumas und Berechnung des Gehaltes an Rohprotein - Teil 2: Getreide, Hülsenfrüchte und gemahlene Getreideerzeugnisse (ISO 16634-2:2016)

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CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

European Foreword

This document (EN ISO 16634-2:2016) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 338 "Cereal and cereal products" the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2016, and conflicting national standards shall be withdrawn at the latest by December 2016.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

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Endorsement notice

The text of ISO 16634-2:2016 has been approved by CEN as EN ISO 16634-2:2016 without any modification.

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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 documents 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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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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 products*, Subcommittee SC 4, *Cereals and pulses*.

This first edition cancels and replaces ISO/TS 16634-2:2009, which has been technically revised.

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*
- *Part 2: Cereals, pulses and milled cereal products*

Introduction

For a long time, the Kjeldahl method has been the most frequently used method for the determination of the protein content of food products. In recent years, the Dumas method has gained importance compared to the Kjeldahl method because it 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 content can be converted into protein content by using an appropriate factor. The value of this factor varies depending on the relative amounts of different proteins and their amino-acid composition in a 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 because 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 protein content of a product calculated by both methods only approximates to the true value, it is a matter of discretion which one is accepted. The best solution is to use 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 the existing factors which indicate the ratio of the protein content 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 2: Cereals, pulses and milled cereal products

1 Scope

This part of ISO 16634 specifies a method for the determination of the total nitrogen content and the calculation of the crude protein content of cereals, pulses and milled cereal products.

This method, like the Kjeldahl method (see References [1] and [6]), does not distinguish between protein nitrogen and non-protein nitrogen. For the calculation of the protein content, various conversion factors are used (see 3.2).

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for 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 712, *Cereals and cereal products — Determination of moisture content — Reference method*

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

ISO 24557, *Pulses — Determination of moisture content — Air-oven method*

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

Note 1 to entry: Determined by the procedure specified in this part of ISO 16634.

Note 2 to entry: The mass fraction is expressed as a percentage.

3.2

crude protein content

nitrogen content (3.1) multiplied by a factor

Note 1 to entry: A 5,7 factor is generally used for cereals for human food (such as wheat, rye and their milled products) and 6,25 for malting barley and cereals for feed and other products falling within the scope of this part of ISO 16634.

Note 2 to entry: The factors for calculation of the crude protein content from the total nitrogen content are derived from the Kjeldahl method, which is the reference method for the determination of total nitrogen content.

4 Principle

Samples are converted into gases by heating in a combustion tube. 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 either [5.1.1](#) or [5.1.2](#).

5.1.1 Carbon dioxide, as pure as possible, but with a minimum CO₂ volume fraction of 99,99 %.

5.1.2 Helium, as pure as possible, but with a minimum He volume fraction of 99,99 %.

5.2 Oxygen, as pure as possible, but with a minimum O₂ volume fraction of 99,99 %.

5.3 Sulfur dioxide and halogen absorbent, to eliminate any sulfur from the sample [e.g. lead chromate (PbCrO₄) or steel wool].

5.4 Copper oxide/platinum catalyst, for the post-combustion tube.

Platinum catalyst [5 % of Pt on alumina (Al₂O₃)] is blended with CuO in the ratio 1 part:7 parts or 1 part:8 parts in accordance with 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, but 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 shall be disaggregated before being inserted into 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 or tungsten (wire, cuttings, turnings or powder), for the reduction tube.

The use of copper or tungsten in one of these forms can improve the precision of analytical results for samples with low nitrogen contents (about 1 % mass fraction).

5.8 Diphosphorus pentoxide (P₂O₅) or granulated magnesium perchlorate [Mg(ClO₄)₂], or another suitable drying agent, 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 (C₄H₇NO₄) or ethylenediaminetetraacetic acid (C₁₀H₁₆N₂O₈) or glutamic acid (C₅H₉NO₄) or hippuric acid (C₉H₉NO₃) standard, or other suitable reference materials with a known, constant, certified nitrogen content.

The minimum recovery should preferably be 99 % mass fraction.

5.13 Light petroleum, with a 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 opening size 800 µm or 1 mm, made of non-ferrous material.

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

NOTE 1 Several instruments provided with an automatic sampler are commercially available.

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

6.5 Dumas apparatus, 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 Schematic diagrams of three commercially available instruments are shown as examples in [Figures B.1](#) to [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 silicaware and glassware carefully and to remove fingerprints from tubes, using a suitable solvent ([5.13](#)), before inserting them into the furnace.

7 Sampling

A representative sample should have been sent to the laboratory. This sample 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 24333 for cereals and cereal products.

8 Preparation of the test sample

The test sample shall be prepared from the laboratory sample in such a way that a homogeneous test sample is obtained.

Using a suitable grinding device ([6.2](#)), grind the laboratory sample. Generally, pass the ground material through a sieve ([6.3](#)) of nominal opening size 800 µm for small sample sizes (under 300 mg) or a sieve of

nominal opening size 1 mm for larger sample sizes (300 mg or more). 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 preferably also be determined when reporting nitrogen or protein contents on a dry-matter or constant-moisture basis. Determination of the moisture content shall be carried out in accordance with ISO 712 for cereals other than maize, ISO 6540 for maize and ISO 24557 for pulses.

The grinder efficiency can be checked by replicate preparation of ground samples of a 2 + 1 mixture of maize 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 carried out 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 ([6.4](#)). For samples low in protein (<1 % mass fraction), the amount of the test portion can be increased up to 3,5 g, depending on the type of Dumas equipment being used and on the nature of the sample.

Depending on the type of equipment used, if the sample contains over 17 % mass fraction of moisture, drying may be necessary before analysis.

Lower test portions may be necessary for very high protein content samples or when only very small amounts of sample are available. In the case of portions below 0,1 g, a second (validation) determination shall be performed.

9.3 Control of oxygen supply

Control the oxygen supply, in particular the flow, in accordance with the instructions of the material supplier.

With each series of nitrogen content determinations, conduct as many blank runs as necessary to stabilize the equipment, using for each run an equivalent mass of sucrose in place of the test portion. The sucrose blank provides the amount of nitrogen that is introduced in the form of atmospheric air trapped within a powdered organic material. Use the mean value of the blank determinations as an error correction in the calculation of the nitrogen content of each test sample.

9.4 Calibration

For instrument calibration, use pure compounds with a known, constant nitrogen content, e.g. aspartic acid (5.12), as standards. Analyse in duplicate, three pure compounds, each in three different amounts chosen as a function of the measurement range for the actual samples.

To prepare a calibration curve, carry out at least five determinations with different amounts of the same compound, choosing the compound and the amounts used in such a way that the curve obtained will cover the range of nitrogen contents in the samples to be analysed.

If the test portion contains more than 200 mg of nitrogen, the calibration curve is likely to be nonlinear. In the nonlinear section, short segments can, nevertheless, be used for calibration purposes. To ensure the reliability of the curve in these segments, the amount of standard used shall be increased in steps corresponding to 1 mg to 5 mg of nitrogen over the segments.

Calibration can also be performed using standard aqueous solutions.

Check the calibration at least three times at the beginning of a series of analyses and then, after every 15 to 25 samples, analysing either one of the standards (5.12) or a sample of known value. The value obtained for the nitrogen mass fraction shall differ by less than 0,05 % from the expected value. If it does not, repeat the calibration check after checking instrument performance.

9.5 Determination

With the instrument operating in the stable state, introduce the test portion in accordance with the manufacturer's instructions.

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

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

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 oxygen is bound to the copper or tungsten (5.7) in the reduction column.

Water is removed by drying tubes filled with magnesium perchlorate, diphosphorus pentoxide or another drying agent (5.8). If carbon dioxide is used as the carrier gas (5.1.1), it is removed by being passed over a suitable absorbent, e.g. sodium hydroxide (5.11), on a suitable support material.

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

The remaining gas mixture, consisting of nitrogen and carrier gas, is passed through a thermal-conductivity detector.

9.6 Detection and data processing

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

10 Calculation and expression of results

10.1 Calculation

10.1.1 Nitrogen content

The results for the total nitrogen content, w_N , expressed as a percentage mass fraction, are usually available in the form of instrument printouts.

10.1.2 Crude protein content

The correction factor, F_c , is obtained from [Formula \(1\)](#):

$$F_c = \frac{100 - w_{H_2O,1}}{100 - w_{H_2O,2}} \quad (1)$$

where

$w_{H_2O,1}$ is the moisture mass fraction, expressed as a percentage, before grinding;

$w_{H_2O,2}$ is the moisture mass fraction, expressed as a percentage, after grinding.

The crude protein content, w_p , expressed as a percentage mass fraction, is obtained from [Formula \(2\)](#):

$$w_p = w_N F F_c \quad (2)$$

where

w_N is the nitrogen content, expressed as a percentage mass fraction, of the sample at its natural moisture content;

F is the generally agreed conversion factor for the product analysed, equal to 5,7 for cereals for human food (such as wheat, rye and their milled products) and 6,25 for malting barley and cereals for feed and other products falling within the scope of this part of ISO 16634 (see [3.2](#)).

If requested, the crude protein content, expressed as a percentage mass fraction of the dry matter, w_{pd} , can be calculated from [Formula \(3\)](#):

$$w_{pd} = \frac{100w_p}{100 - w_{H_2O}} \quad (3)$$

where w_{H_2O} is the moisture content, expressed as a percentage mass fraction, determined in accordance with ISO 712, ISO 6540 or ISO 24557.

10.2 Expression of results

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

11 Precision

11.1 Interlaboratory tests

Details of interlaboratory tests carried out to determine the precision of the method are given in [Annex D](#).

The values derived from these interlaboratory tests may not be applicable to concentration ranges and matrices other than those given, i.e. to nitrogen contents between 0,05 % and 13,89 %.

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 not be greater than the repeatability limit, r , given below in more than 5 % of cases:

$$r = 2,8 s_r = 2,8 (0,0013 w_N + 0,012) \quad (4)$$

where

s_r is the repeatability standard deviation;

w_N is the nitrogen content, expressed as a percentage mass fraction, of the sample at its natural moisture content.

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 not be greater than the repeatability limit, R , given below in more than 5 % of cases:

$$R = 2,8 s_R = 2,8 (0,0126 w_N + 0,017) \quad (5)$$

where

s_R is the reproducibility standard deviation;

w_N is the nitrogen content, expressed as a percentage mass fraction.

11.4 Critical difference

11.4.1 Comparison of two groups of measurements in the same laboratory

The critical difference, CD, i.e. the difference between two averaged values obtained from two test results under repeatability conditions, is given by [Formula \(6\)](#).

$$CD = 2,8 s_r \sqrt{\frac{1}{2n_1} + \frac{1}{2n_2}} = 2,8 s_r \sqrt{\frac{1}{2}} = 1,98 s_r \quad (6)$$

where

s_r is the repeatability standard deviation;

n_1 and n_2 are the number of test results corresponding to each of the averaged values.

11.4.2 Comparison of two groups of measurements in two different laboratories

The critical difference between two averaged values obtained in two different laboratories from two test results under repeatability conditions is equal to:

$$CD = 2,8 \sqrt{s_R^2 - s_r^2 \left(1 - \frac{1}{2n_1} - \frac{1}{2n_2}\right)} = 2,8 \sqrt{s_R^2 - 0,5s_r^2} \quad (7)$$

where

s_R is the reproducibility standard deviation;

s_r is the repeatability standard deviation;

n_1 and n_2 are the number of test results corresponding to each of the averaged values.

11.5 Uncertainty

The measurement uncertainty, U_e , is a parameter representing the distribution of the values that may reasonably be attributed to the result. This uncertainty is given by a statistical distribution of the results from the interlaboratory tests and is characterized by the experimental standard deviation.

The uncertainty, U_e , is equal to plus or minus twice the reproducibility standard deviation given in this part of ISO 16634.

$$U_e = \pm 2 s_R \quad (8)$$

where s_R is the reproducibility standard deviation.

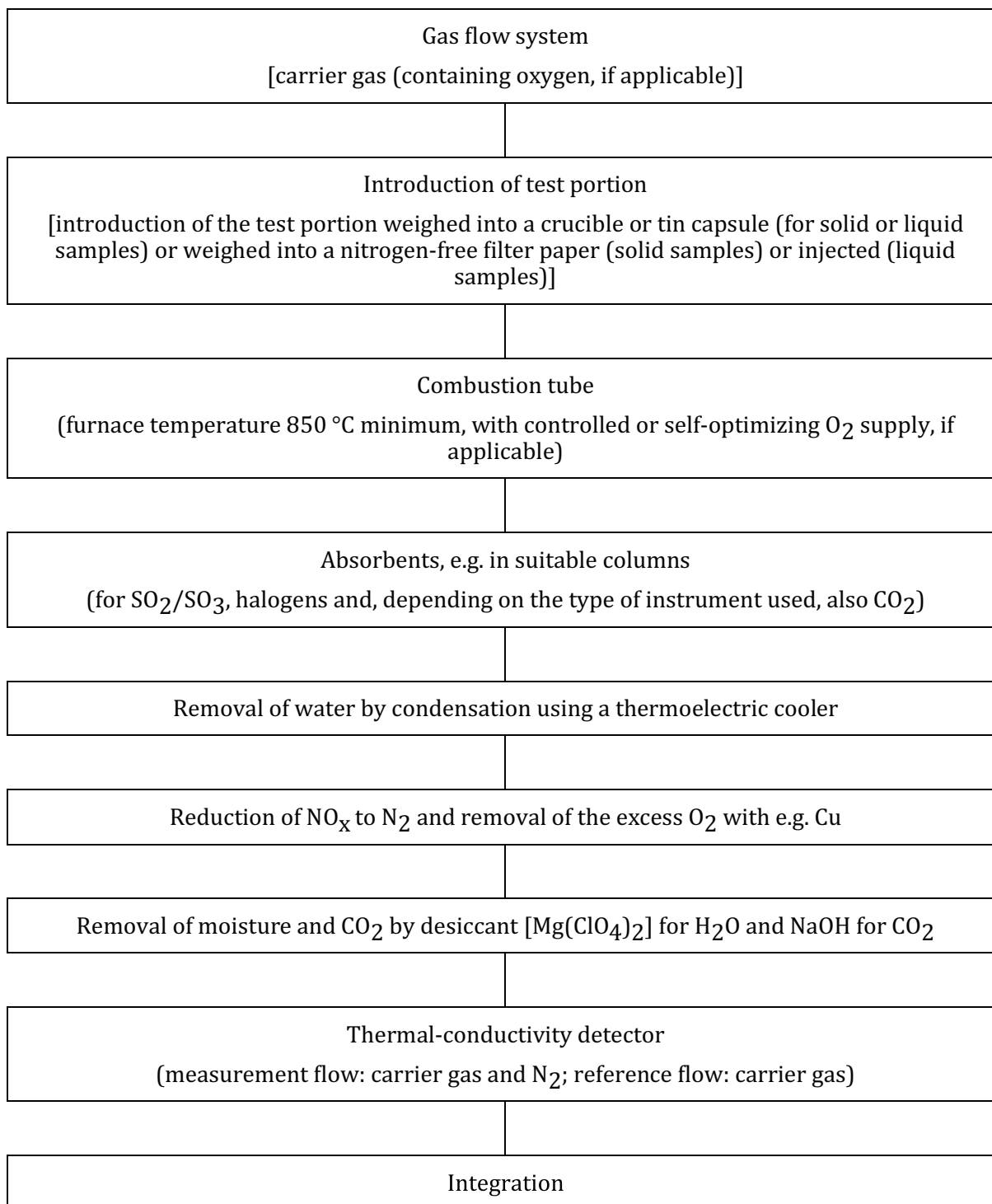
12 Test report

The test report shall contain at least the following information:

- 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 part of ISO 16634, i.e. ISO 16634-2;
- d) all operating details not specified in this part of ISO 16634, or regarded as optional, together with details of any incident which may have influenced the test result(s);
- e) the test result(s) obtained, the conversion factor used and the moisture content of the test sample or the reference moisture content;
- f) if the repeatability has been checked, the final quoted result obtained;
- g) the date of the test.

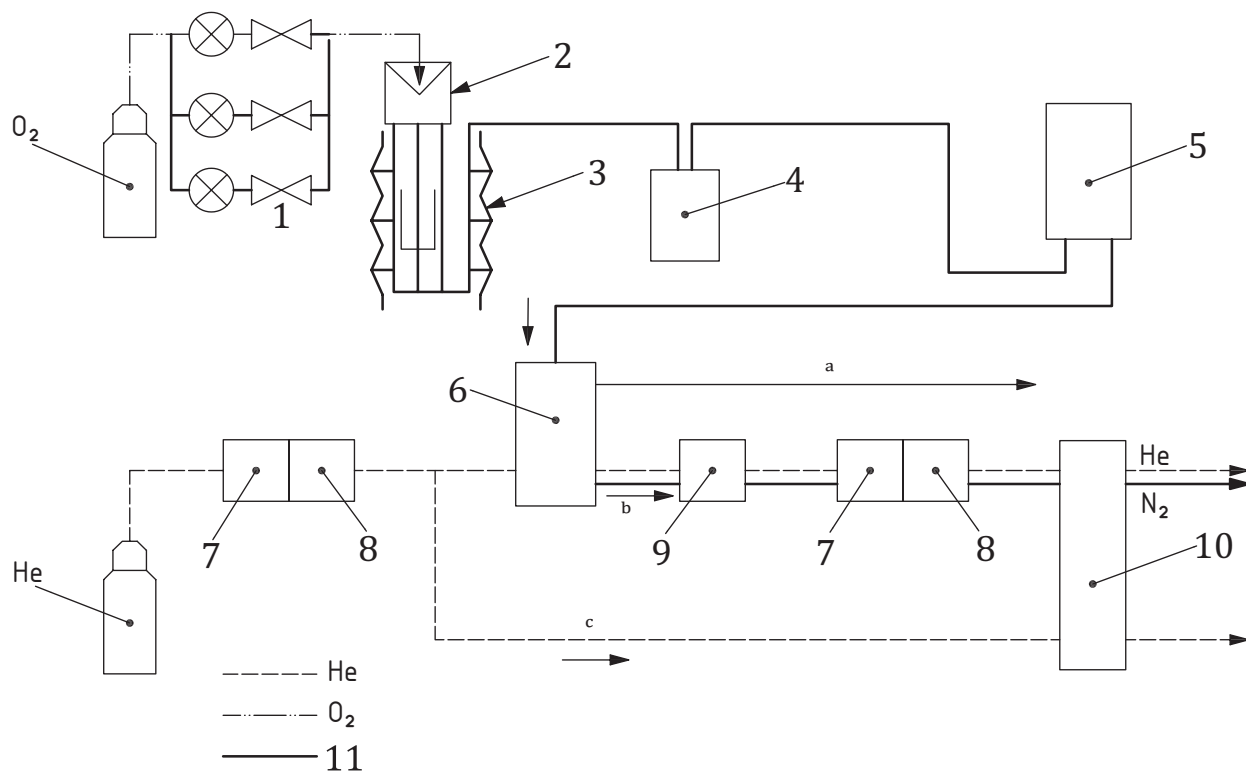
Annex A (informative)

Flowchart for a basic Dumas apparatus



Annex B (informative)

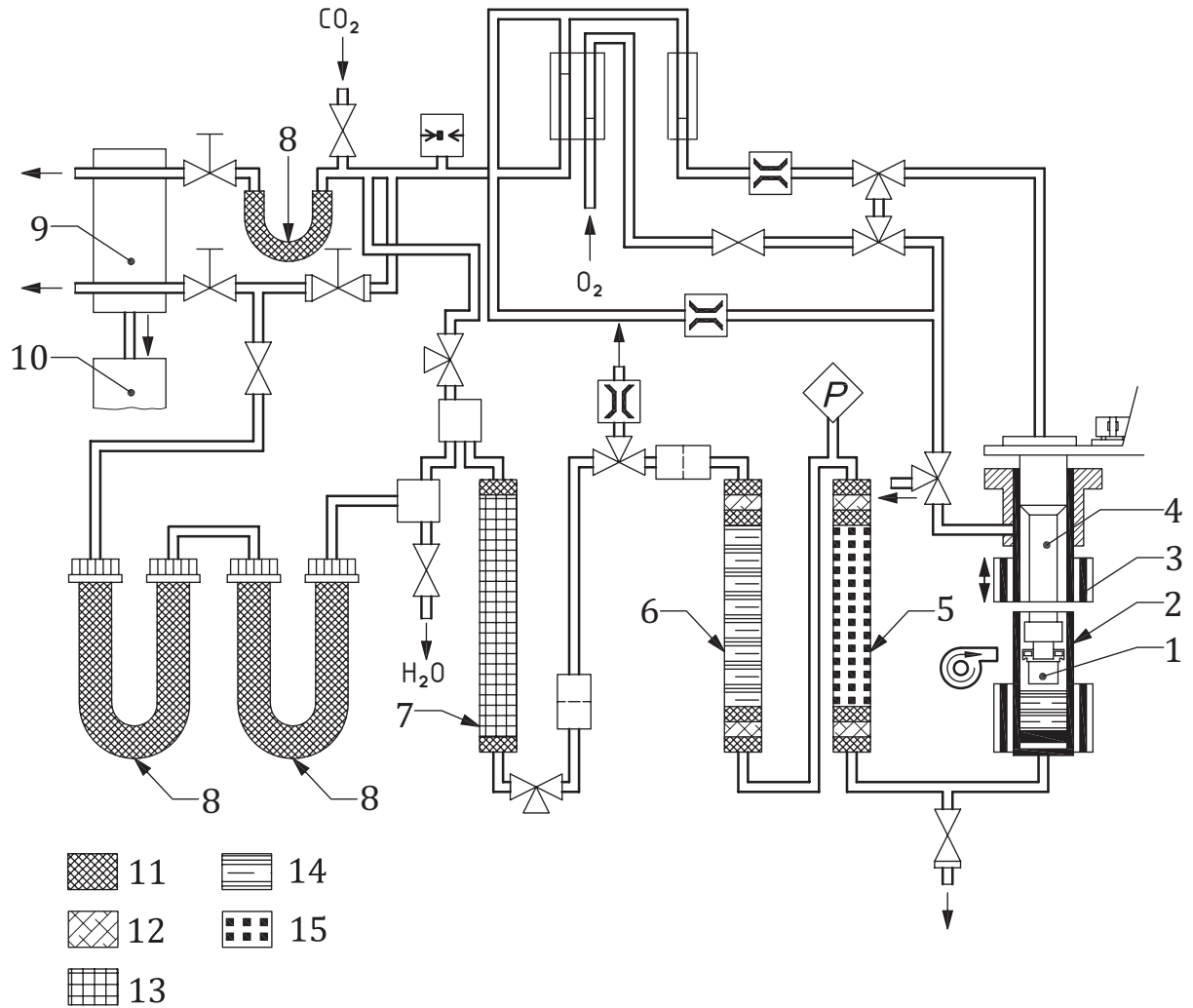
Schematic diagrams of suitable types of Dumas apparatus



Key

- | | |
|--|---|
| 1 oxygen flow controller | 8 magnesium perchlorate |
| 2 introduction of test portion | 9 copper catalyst (reduces NO _x and O ₂) |
| 3 resistance furnace with crucible | 10 thermal-conductivity detector |
| 4 (thermoelectric) cooler | 11 combustion gases containing N ₂ |
| 5 mixing container/ballast column | a Surplus combustion gases. |
| 6 dosing device | b Measurement flow. |
| 7 sodium hydroxide on support material | c Reference flow. |

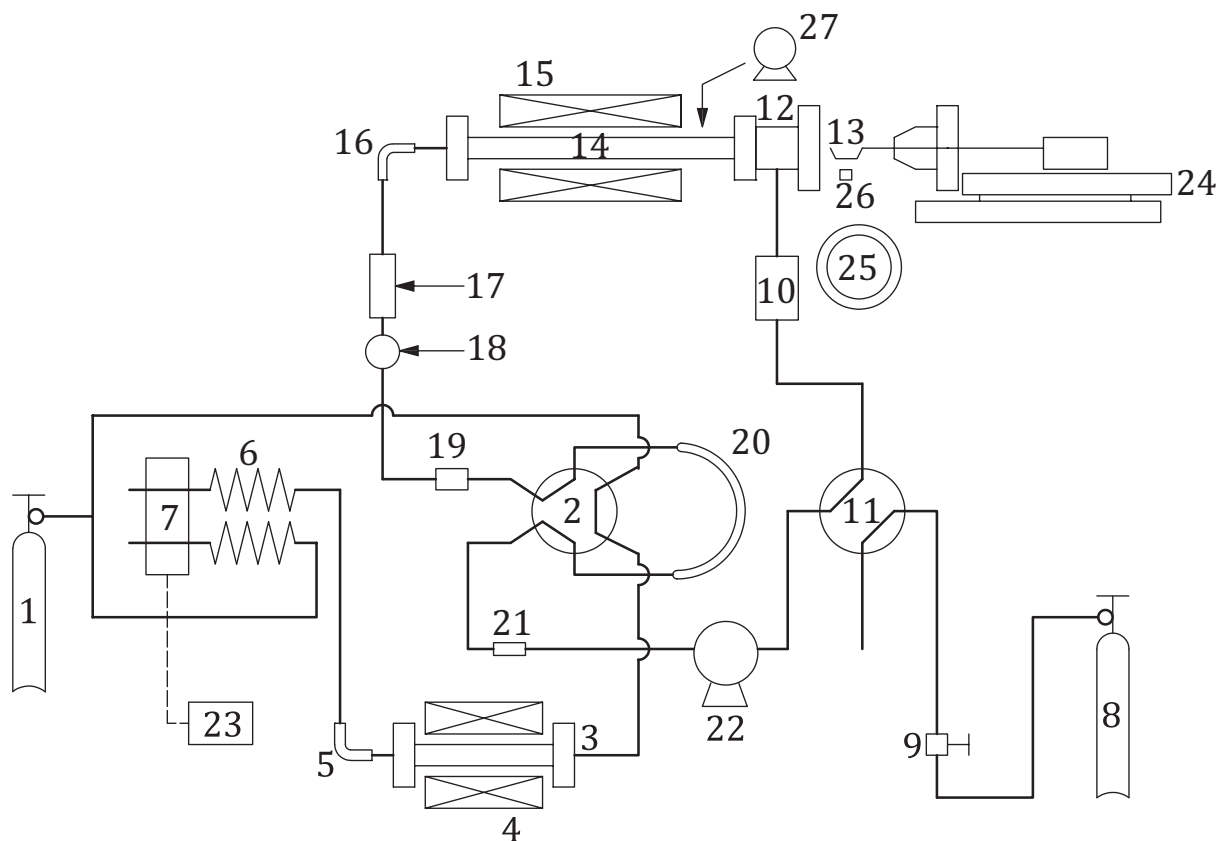
Figure B.1 — First example of a Dumas apparatus (carrier gas He)



Key

- | | | | |
|---|---------------------------------|----|------------------------------------|
| 1 | test crucible | 9 | thermal-conductivity detector |
| 2 | combustion column | 10 | integrator |
| 3 | combustion furnace (mobile) | 11 | drying agent |
| 4 | crucible holder | 12 | silver wool |
| 5 | SO ₂ absorption tube | 13 | copper wire |
| 6 | post-combustion tube | 14 | copper wire with platinum catalyst |
| 7 | reduction column | 15 | lead chromate |
| 8 | drying tube | | |

Figure B.2 — Second example of a Dumas apparatus (carrier gas CO₂)



Key

- | | | | |
|----|-------------------------------|----|--|
| 1 | helium cylinder | 15 | reaction furnace |
| 2 | valve | 16 | tube for checking completeness of combustion |
| 3 | reduction tube | 17 | condenser for removing water vapour |
| 4 | reduction furnace | 18 | gas-mixing tube |
| 5 | gas absorption tube | 19 | filter no. 1 |
| 6 | gas separator column | 20 | measurement tube |
| 7 | thermal-conductivity detector | 21 | filter no. 2 |
| 8 | oxygen cylinder | 22 | circulation pump |
| 9 | oxygen flow controller | 23 | data processor |
| 10 | flow meter | 24 | test portion insertion device |
| 11 | valve | 25 | test portion tray |
| 12 | test portion inlet | 26 | lifting device for test portion tray |
| 13 | test portion insertion shaft | 27 | cooling-air pump |
| 14 | reaction tube | | |

Figure B.3 — Third example of a Dumas apparatus (carrier gas He)

Annex C (informative)

Equipment calibration

C.1 Calibration compounds

Some of the instruments available require entry of the expected oxygen demand. [Table C.1](#) gives the reference values of some calibration compounds needed for the calculations presented in [C.2](#).

The calculations in [C.2](#) are necessary for some types of instrument (those involving a moderate O₂ surplus in the presence of CO₂ as carrier gas). All calculations are based on the assumption that the sample consists only of the elements C, N, H and O.

Table C.1 — Oxygen demand of pure compounds suitable for calibration of the equipment

Compound	Nitrogen content % mass fraction	Maximum theoretical oxygen demand ml/g	Empirical oxygen demand ml/g
Urea	46,65	1 305	560
Aspartic acid	10,53	800	631
Tyrosine	7,73	1 391	1 267
Glutamic acid	9,52	952	800
Phenylalanine	8,48	1 593	1 458
Ethylenediamine- tetraacetic acid	9,59	920	767
Hippuric acid	7,82	1 344	1 219

C.2 Examples for calculation of the estimated oxygen demand

C.2.1 Example 1

Urea (H₂NCONH₂): 1 mol corresponds to 60,06 g; mass of test portion 1 000 mg.

The 1 000 mg test portion of urea therefore contains the following:

- 199,8 mg of C;
- 66,6 mg of H;
- 466,5 mg of N;
- 266,4 mg of O.

The amount of oxygen required for complete combustion to carbon dioxide and water is calculated taking into account the oxygen content of the compound and the following facts:

- a) the molar volume of an ideal gas is 22,4 l (at $T = 0\text{ °C}$ and $P = 0,1\text{ MPa}$);
- b) 1 mol of C corresponds to 12 g (12 000 mg);
- c) 1 mol of H₂ corresponds to 2 g (2 000 mg);

d) 1 mol of N_2 corresponds to 28 g (28 000 mg);

e) 1 mol of O_2 corresponds to 32 g (32 000 mg).

As a result, 1 305 ml of oxygen are needed for the combustion of 1 g of urea.

C.2.2 Example 2

Aspartic acid [$HO_2CCH_2CH(NH_2)CO_2H$]: 1 mol corresponds to 133,10 g, mass of test portion 1 000 mg.

The 1 000 mg test portion of aspartic acid therefore contains the following:

— 360,6 mg of C;

— 52,6 mg of H;

— 105,2 mg of N;

— 480,8 mg of O.

The amount of oxygen required for complete combustion to carbon dioxide and water is calculated taking into account the oxygen content of the compound and the following facts:

a) the molar volume of an ideal gas is 22,4 l (at $T = 0\text{ }^\circ\text{C}$ and $P = 0,1\text{ MPa}$);

b) 1 mol of C corresponds to 12 g (12 000 mg);

c) 1 mol of H_2 corresponds to 2 g (2 000 mg);

d) 1 mol of N_2 corresponds to 28 g (28 000 mg);

e) 1 mol of O_2 corresponds to 32 g (32 000 mg).

As a result, 800 ml of oxygen are needed for the combustion of 1 g of aspartic acid.

Annex D (informative)

Results of interlaboratory tests

D.1 General

The values of the repeatability limit and reproducibility limit for this method have been derived from the results of an international interlaboratory test programme carried out in accordance with ISO 5725-1, ISO 5725-2 and ISO 5725-6.

The tests were carried out on 13 samples of cereals and pulses. 17 laboratories in 6 countries took part.

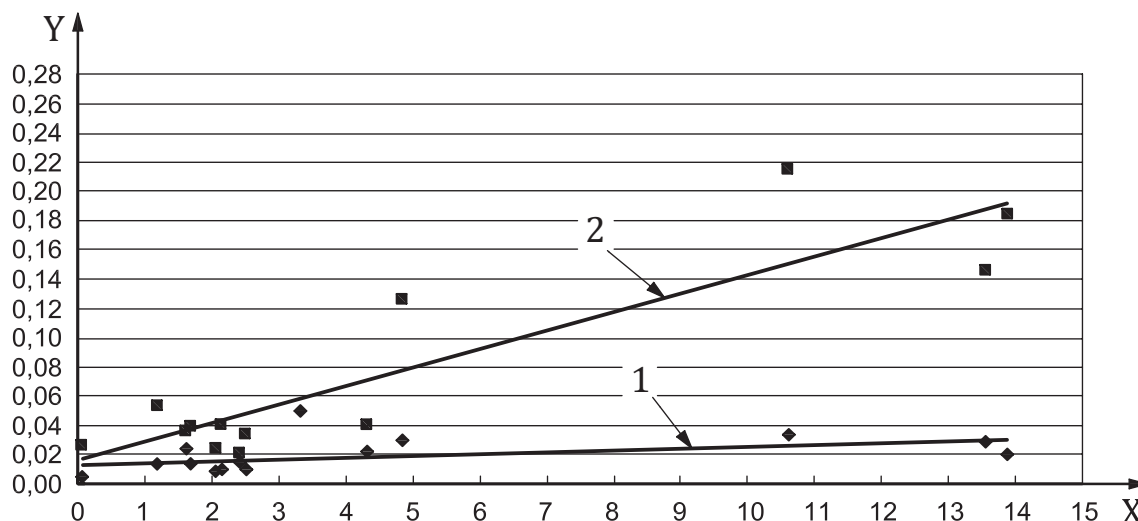
This test programme was organized by ARVALIS-Institut du Végétal in June 2007.

The results obtained were subjected to statistical analysis in accordance with ISO 5725-1, ISO 5725-2 and ISO 5725-6 to give the precision data shown in [Tables D.1 to D.3](#) and [Figure D.1](#) for nitrogen content and in [Tables D.4 to D.6](#) and [Figure D.2](#) for protein content.

D.2 Precision data for nitrogen content

Table D.1 — Interlaboratory test results for nitrogen content

Parameter	Starch	Maize	Rye	Barley	Common wheat flour	Common wheat	Durum wheat semolina	Durum wheat	Pea	Horse bean	Maize gluten	Common wheat gluten	Pea proteins
Number of laboratories or tests (after elimination of abnormal data)	15	16	16	16	14	15	15	17	17	17	17	17	17
Average nitrogen content, w_N (%)	0,05	1,18	1,63	1,68	2,07	2,16	2,42	2,51	4,33	4,85	10,61	13,55	13,89
Repeatability standard deviation, s_r (%)	0,004 6	0,013 9	0,023 4	0,013 9	0,008	0,010 1	0,015 1	0,010 1	0,022 3	0,031	0,034 5	0,029 4	0,020 3
Repeatability coefficient of variation, $CV(r)$ (= s_r/w_N) (%)	0,094	0,012	0,014	0,008	0,004	0,005	0,006	0,004	0,005	0,006	0,003	0,002	0,001
Repeatability limit, r (= $2,8s_r$)	0,01	0,04	0,06	0,04	0,02	0,03	0,04	0,03	0,06	0,09	0,10	0,08	0,06
Reproducibility standard deviation, s_R (%)	0,027	0,053	0,037	0,039	0,024	0,040	0,022	0,034	0,040	0,126	0,215	0,146	0,184
Reproducibility coefficient of variation, $CV(R)$ (= s_R/w_N) (%)	0,543	0,045	0,023	0,023	0,011	0,019	0,009	0,014	0,009	0,026	0,020	0,011	0,013
Reproducibility limit, R (= $2,8s_R$)	0,07	0,15	0,10	0,11	0,07	0,11	0,06	0,09	0,11	0,35	0,59	0,40	0,51



Key

- X nitrogen content (% by mass)
- Y standard deviation in nitrogen content
- 1 repeatability standard deviation
- 2 reproducibility standard deviation

Figure D.1 — Relationship between the repeatability and reproducibility standard deviations and the nitrogen content

The graph (see [Figure D.1](#)) shows that the repeatability and reproducibility values increase (i.e. the precision decreases) with increasing nitrogen content.

Table D.2 — Summary of precision data for nitrogen content

Parameter	Range	Relationship	Repeatability	Reproducibility
Nitrogen content (% by mass on a dry-matter basis)	from 0,05 to 13,89	<i>r</i> : linear <i>R</i> : linear	$s_r = 0,001\ 3\ w_N + 0,012$	$s_R = 0,012\ 6\ w_N + 0,017$
			Correlation coefficient $R^2 = 0,452\ 9$	Correlation coefficient $R^2 = 0,797\ 6$

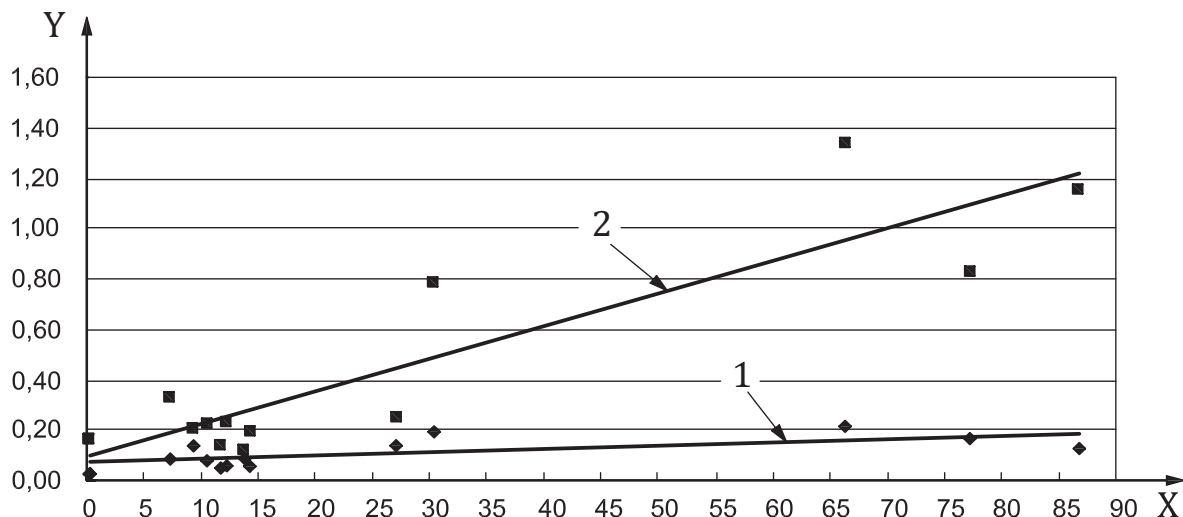
Table D.3 — Example of a practical application of the precision data for nitrogen content

Nitrogen content %	Repeatability standard deviation s_r	Repeatability limit r	Reproducibility standard deviation s_R	Reproducibility limit R	Critical difference between two means	
					Within one lab CD(r)	Between two laboratories CD(R)
0,05	0,012	0,03	0,018	0,05	0,02	0,04
0,50	0,013	0,04	0,023	0,06	0,03	0,06
1,00	0,013	0,04	0,030	0,08	0,03	0,08
2,00	0,015	0,04	0,042	0,12	0,03	0,11
3,00	0,016	0,04	0,055	0,15	0,03	0,15
4,00	0,017	0,05	0,067	0,19	0,03	0,18
5,00	0,019	0,05	0,080	0,22	0,04	0,22
6,00	0,020	0,05	0,093	0,26	0,04	0,25
7,00	0,021	0,06	0,105	0,29	0,04	0,29
8,00	0,022	0,06	0,118	0,33	0,04	0,32
9,00	0,024	0,07	0,130	0,36	0,05	0,36
10,00	0,025	0,07	0,143	0,40	0,05	0,39
11,00	0,026	0,07	0,156	0,43	0,05	0,43
12,00	0,028	0,08	0,168	0,47	0,05	0,46
13,00	0,029	0,08	0,181	0,50	0,06	0,50
13,85	0,030	0,08	0,192	0,53	0,06	0,53

D.3 Precision data for protein content

Table D.4 — Interlaboratory test results for protein content

Parameter	Starch	Maize	Rye	Barley	Common wheat flour	Common wheat	Durum wheat semolina	Durum wheat	Pea	Horse bean	Maize gluten	Common wheat gluten	Pea pro-teins
Conversion factor	6,25	6,25	5,7	6,25	5,7	5,7	5,7	5,7	6,25	6,25	6,25	5,7	6,25
Number of laboratories or tests (after elimination of abnormal data)	15	16	16	16	14	15	15	17	17	17	17	17	17
Average protein content, w_{pd} (%)	0,31	7,38	9,29	10,50	11,80	12,31	13,79	14,31	27,06	30,31	66,31	77,24	86,81
Repeatability standard deviation, s_r (%)	0,029	0,087	0,134	0,079	0,047	0,057	0,086	0,058	0,139	0,192	0,216	0,168	0,127
Repeatability coefficient of variation, $CV(r)$ ($= s_r/w_{pd}$) (%)	0,093	0,012	0,014	0,008	0,004	0,005	0,006	0,004	0,005	0,006	0,003	0,002	0,001
Repeatability limit, r ($= 2,8 s_r$)	0,08	0,24	0,37	0,22	0,13	0,16	0,24	0,16	0,39	0,53	0,60	0,47	0,35
Reproducibility standard deviation, s_R (%)	0,167	0,330	0,212	0,223	0,136	0,230	0,124	0,194	0,250	0,786	1,342	0,831	1,152
Reproducibility coefficient of variation, $CV(R)$ ($= s_R/w_{pd}$) (%)	0,534	0,045	0,023	0,021	0,012	0,019	0,009	0,014	0,009	0,026	0,020	0,011	0,013
Reproducibility limit, R ($= 2,8 s_R$)	0,46	0,91	0,59	0,62	0,38	0,64	0,34	0,54	0,69	2,18	3,72	2,30	3,19



Key

- X protein content (% by mass)
- Y standard deviation in protein content
- 1 repeatability standard deviation
- 2 reproducibility standard deviation

Figure D.2 — Relationship between the repeatability and reproducibility standard deviations and the protein content

Table D.5 — Summary of precision data for protein content

Parameter	Range	Relationship	Repeatability	Reproducibility
Protein content (% by mass on a dry-matter basis)	from 0,3 to 86,8	<i>r</i> : linear <i>R</i> : linear	$s_r = 0,001\ 4w_{pd} + 0,070\ 6$	$s_R = 0,012\ 9w_{pd} + 0,094\ 5$
			Correlation coefficient $R^2 = 0,458$	Correlation coefficient $R^2 = 0,801$

Table D.6 — Example of a practical application of the precision data for protein content

Protein content %	Repeatability standard deviation s_r	Repeatability limit r	Reproducibility standard deviation s_R	Reproducibility limit R	Critical difference between two means	
					Within one lab CD(r)	Between two laboratories CD(R)
0,35	0,070	0,19	0,104	0,29	0,14	0,25
5,0	0,075	0,21	0,160	0,44	0,15	0,42
10,0	0,081	0,22	0,220	0,61	0,16	0,59
15,0	0,087	0,24	0,280	0,77	0,17	0,76
20,0	0,093	0,26	0,340	0,94	0,18	0,92
25,0	0,099	0,27	0,400	1,11	0,20	1,09
30,0	0,105	0,29	0,460	1,27	0,21	1,26
35,0	0,111	0,31	0,520	1,44	0,22	1,42
40,0	0,117	0,32	0,580	1,61	0,23	1,59
45,0	0,123	0,34	0,640	1,77	0,24	1,76
50,0	0,129	0,36	0,700	1,94	0,26	1,92
55,0	0,135	0,37	0,760	2,10	0,27	2,09
60,0	0,141	0,39	0,820	2,27	0,28	2,25
65,0	0,147	0,41	0,880	2,44	0,29	2,42
70,0	0,153	0,42	0,940	2,60	0,30	2,59
75,0	0,159	0,44	1,000	2,77	0,32	2,75
80,0	0,165	0,46	1,060	2,94	0,33	2,92
85,0	0,171	0,47	1,120	3,10	0,34	3,08
86,8	0,173	0,48	1,141	3,16	0,34	3,14

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