

खाद्य उत्पाद — डुमास सिद्धान्त अनुसार  
दहन द्वारा कुल नाइट्रोजन की मात्रा का  
निर्धारण और कच्चे प्रोटीन की मात्रा की  
गणना

भाग 1 तिलहन और पशु आहार सामग्री

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

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## NATIONAL FOREWORD

This Indian Standard which is identical to ISO 16634-1 : 2008 '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' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on recommendation of the Oils and Oilseeds Sectional Committee and approval of the Food and Agriculture Division Council.

This standard is published in two 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'. ISO 16634-2 : 2016 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 and has been identically adopted as Indian Standard under single numbering system (IS/ISO 16634-2 : 2016).

The text of ISO standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

- a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'; and
- b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to the following International Standards for which Indian Standards also exist. The corresponding Indian Standard which is to be substituted in its place is listed below along with its degree of equivalence for the edition indicated.

<i>International Standard</i>	<i>Corresponding Indian Standard</i>	<i>Degree of Equivalence</i>
ISO 6498 Animal feeding stuffs — Preparation of test samples	IS 14831 : 2021/ISO 6498 : 2012 Animal feeding stuffs — Guidelines for sample preparation ( <i>first revision</i> )	Identical
ISO 6496 Animal feeding stuffs — Determination of moisture and other volatile matter content	IS/ISO 6496 : 1999 Animal feeding stuffs — Determination of moisture and other volatile matter content	Identical

The Committee has reviewed the provisions of the following International Standards referred in the adopted standard and has decided that they are acceptable for use in conjunction with these standards:

<i>International Standard</i>	<i>Title</i>
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

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'.

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## 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.

*Indian Standard*

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<sup>[10]</sup>.

**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,  $\varphi(\text{CO}_2) \geq 99,99 \%$ .

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

**5.2 Oxygen**, as pure as possible and of minimum volume fraction,  $\varphi(\text{O}_2) \geq 99,99 \%$ .

**5.3 Sulfur dioxide and halogen absorbent**, to eliminate any sulfur from the sample [e.g. lead chromate ( $\text{PbCrO}_4$ ) or steel wool].

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

Platinum catalyst [5 % of Pt on alumina ( $\text{Al}_2\text{O}_3$ )] 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 ( $\text{P}_2\text{O}_5$ ) or granulated magnesium perchlorate [ $\text{Mg}(\text{ClO}_4)_2$ ]**, 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<sub>4</sub>H<sub>7</sub>NO<sub>4</sub>) or **ethylenediaminetetraacetic acid** (C<sub>10</sub>H<sub>16</sub>N<sub>2</sub>O<sub>8</sub>) or **glutamic acid** (C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>) or **hippuric acid** (C<sub>9</sub>H<sub>9</sub>NO<sub>3</sub>) **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**<sup>1)</sup>, 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.

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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<sup>[1]</sup> for oilseeds, in ISO 5500<sup>[3]</sup> 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)<sup>[15]</sup>. 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)].

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 percentage mass fraction, are usually available from instrument printouts.

#### 10.1.2 Crude protein content

The correction factor,  $F_c$ , is obtained by using Equation (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 by using Equation (2):

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

where

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

$F$  is the generally agreed ratio for the analysed product, equal to 6,25 for animal feeding stuffs (see Annex D).

On request, the crude protein content,  $w_{pd}$ , expressed as a percentage mass fraction of the dry matter, can be calculated by using Equation (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 according to ISO 665, ISO 771 or ISO 6496.

## 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) 0,1 % mass fraction if the sample contains less than 4 % mass fraction nitrogen; and
- b) 2 % mass fraction of the nitrogen content if the sample contains 4 % mass fraction 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 than:

- a) a mass fraction of 0,17 % if the sample contains less than a mass fraction of 4 % nitrogen; and
- b) a mass fraction of 4 % of the nitrogen content if the sample contains a mass fraction of 4 % or more of nitrogen.

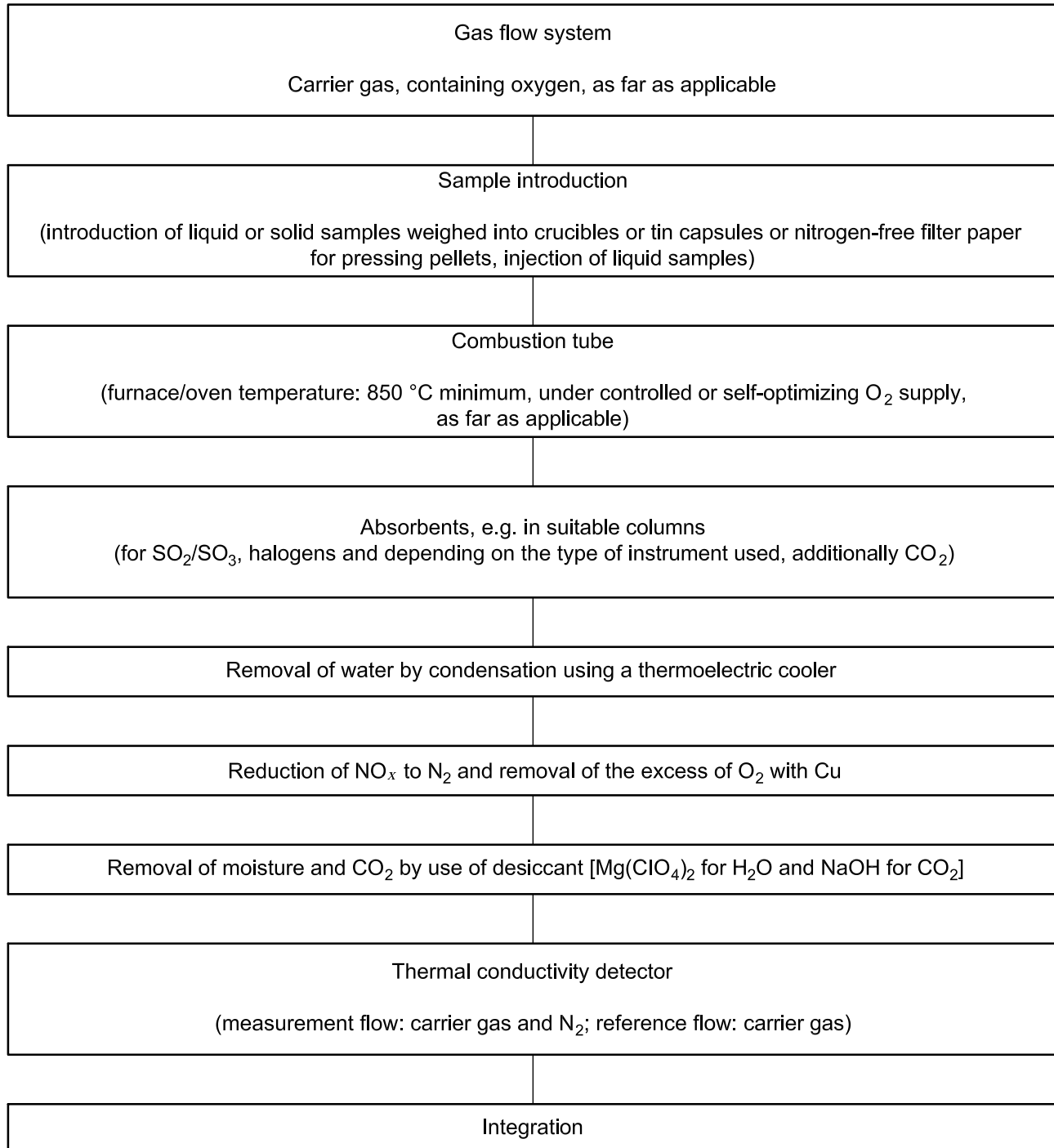
## 12 Test report

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;
- 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.

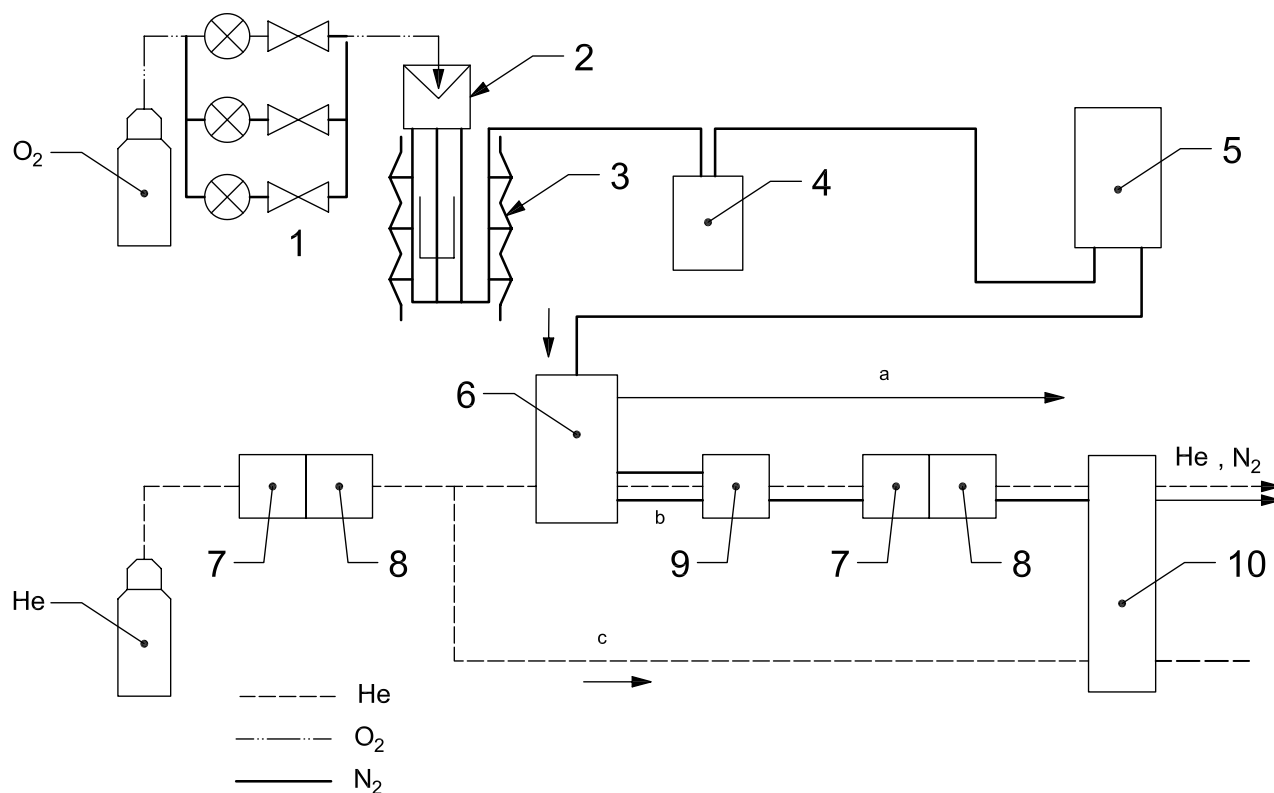
## Annex A (informative)

### Flowchart for the basic design of a Dumas apparatus



**Annex B**  
 (informative)

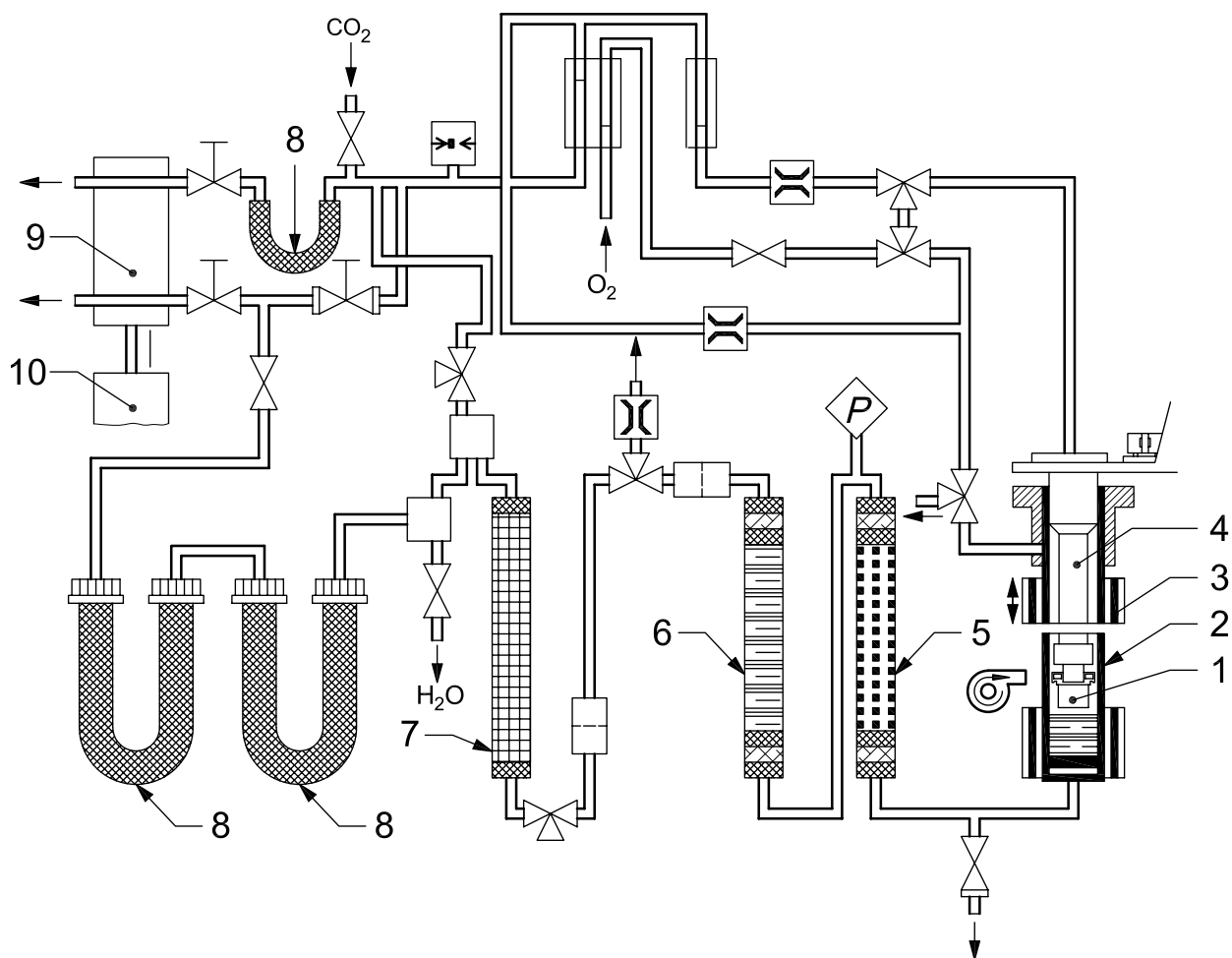
**Schemes of suitable types of Dumas apparatus**








**Key**

- 1 oxygen flow control
  - 2 sample loader
  - 3 resistance furnace with crucible
  - 4 (thermoelectric) cooler
  - 5 mixing container (ballast column)
  - 6 aliquot doser
  - 7 sodium hydroxide on support material
  - 8 magnesium perchlorate
  - 9 copper catalyst (reduces  $\text{NO}_x$  and  $\text{O}_2$ )
  - 10 thermal conductivity detector
- a Surplus of combustion gases.  
 b Measuring flow.  
 c Reference flow.

**Figure B.1 — Dumas apparatus, example 1 (carrier gas: helium)**

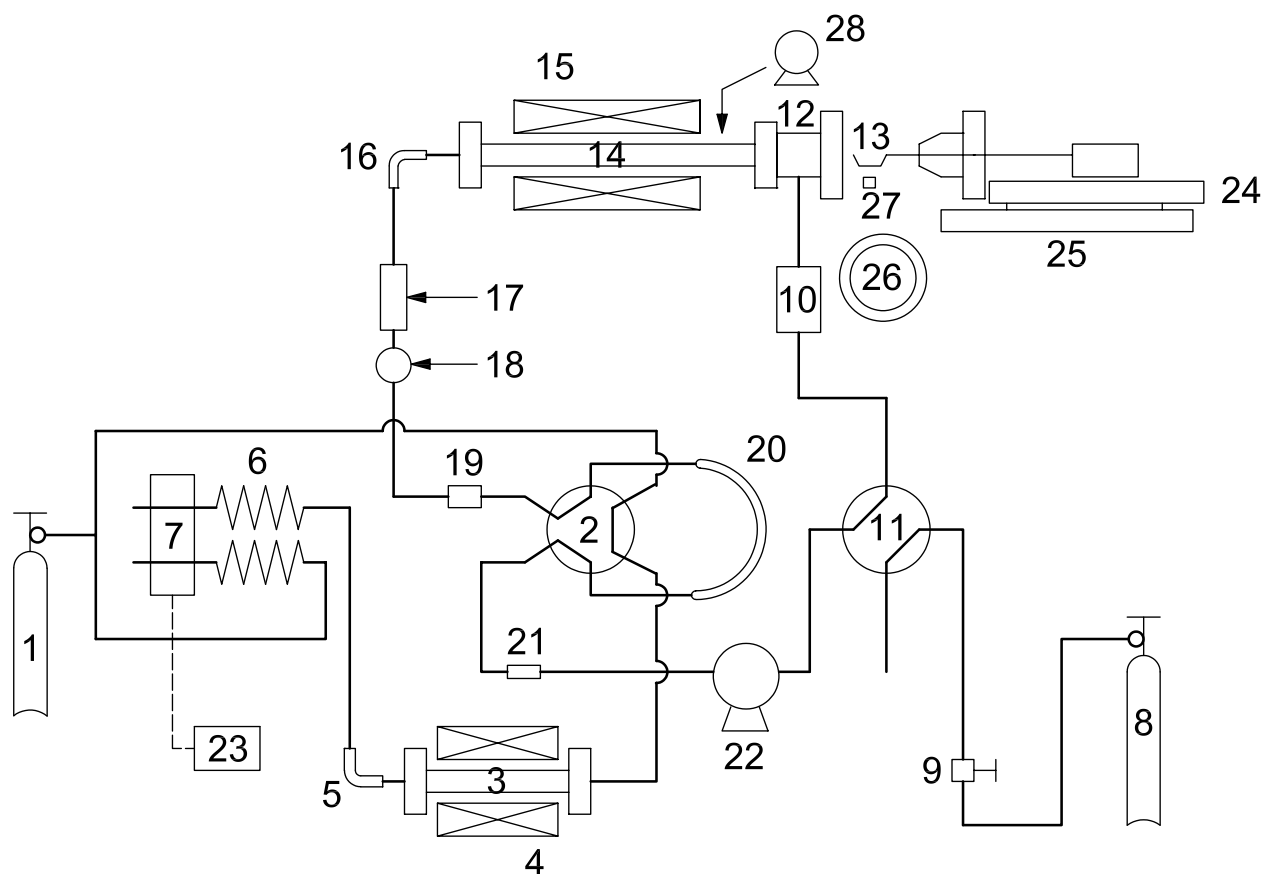


-  drying agent
-  silver wool
-  copper wire
-  copper wire and platinum catalyst
-  lead chromate

**Key**

- |                                   |                                 |
|-----------------------------------|---------------------------------|
| 1 test crucible                   | 6 post-column tube              |
| 2 combustion column               | 7 reduction column              |
| 3 combustion furnace (mobile)     | 8 drying tube                   |
| 4 crucible holder                 | 9 thermal conductivity detector |
| 5 SO <sub>2</sub> absorption tube | 10 integrator                   |

**Figure B.2 —Dumas apparatus, example 2 (carrier gas: carbon dioxide)**



**Key**

- |    |                               |    |   |
|----|-------------------------------|----|---|
| 1  | helium cylinder               | 15 | reaction furnace                        |
| 2  | valve                         | 16 | combustion checking tube                |
| 3  | reduction tube                | 17 | condenser for dehydration               |
| 4  | reduction furnace             | 18 | gas mixing tube                         |
| 5  | gas absorption tube           | 19 | filter No.1                             |
| 6  | gas separator column          | 20 | measuring tube                          |
| 7  | thermal conductivity detector | 21 | filter No.2                             |
| 8  | oxygen cylinder               | 22 | circulation tube                        |
| 9  | mass flow controller          | 23 | data processor                          |
| 10 | flow meter                    | 24 | air cylinder block for sample insertion |
| 11 | valve                         | 25 | air cylinder block for sample insertion |
| 12 | sample inlet                  | 26 | sample tray                             |
| 13 | sample insertion shaft        | 27 | lift of sample tray                     |
| 14 | reaction tube                 | 28 | cooling air pump                        |

**Figure B.3 —Dumas apparatus, example 3 (carrier gas: helium)**

## Annex C (informative)

### Equipment calibration

#### C.1 Calibration compounds

Some of the instruments available require entry of the expected oxygen demand.

The calculations in Clause C.2 are necessary for some types of instruments (moderate molecular oxygen surplus in the presence of carbon dioxide as carrier gas). All calculations proceed on the assumption that the samples consist only of the elements carbon, nitrogen, hydrogen, and oxygen.

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

Compound	Nitrogen content % mass fraction	Maximum of 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
Ethylenediaminetetraacetic 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 ( $\text{CH}_4\text{N}_2\text{O}$ ): 1 mol corresponds to 60,06 g, sample mass 1 000 mg.

Therefore, 1 000 mg of urea contain

- 199,8 mg of C,
- 66,6 mg of H,
- 466,5 mg of N, and
- 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  $T = 0\text{ }^\circ\text{C}$  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, 1 305 ml of oxygen are needed for combustion of 1 g of urea.

### C.2.2 Example 2

Aspartic acid [ $C_4H_7NO_4$ ]: 1 mol corresponds to 133,10 g, sample mass 1 000 mg.

Therefore, 1 000 mg of aspartic acid contain

- 360,6 mg of C,
- 52,6 mg of H,
- 105,2 mg of N, and
- 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 combustion of 1 g of aspartic acid.

## Annex D (informative)

### Examples of factors for converting nitrogen content to protein content <sup>2)</sup>

The general conversion factor for animal feeding stuffs is 6,25. For other commodities, other factors may apply.

Commodity	Nitrogen to protein conversion		
	Reference [19]	Reference [35]	Reference [37]
Barley	—	5,68	5,83
Buckwheat	—	5,53	—
Coconut meat (raw)	5,3	—	5,30
Corn, flour	6,25	—	—
Cottonseed (roasted), flour	5,3	—	—
Flax meal	—	5,41	—
Millet (raw)	5,83	5,68	—
Mustard meal	—	5,4	—
Oats	—	5,5	—
Oats (oatmeal, rolled oats)	—	—	5,83
Peanuts (dried), flour	5,46	—	—
Rapeseed meal	—	5,53	—
Rice (brown, long grain)	5,95	—	—
Rice home-pounded, undermilled, parboiled	—	—	5,95
Rice husked or brown (only hulls removed)	—	—	5,95
Rice milled, white	—	—	5,95
Rye, dark flour	—	5,64	5,83
Safflower meal	5,3	—	—
Safflower seed (dried)	5,3	—	5,3
Sesame (dried)	5,3	—	5,3
Soya bean (roasted), flour	5,71	—	—
Soya bean, seeds, flour or products	—	—	5,71
Sunflower flour	5,3	—	—
Sunflower seed (dried)	5,3	—	5,3
Triticale	—	5,76	—
Wheat (hard red)	5,83	5,61	—
Wheat bran	—	5,26	6,31
Wheat germ	—	5,45	—
Wheat wholemeal or flour or bulgur	—	—	5,83

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 this 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.

## **Annex E** (informative)

### **Result of collaborative studies**

The results are listed in Table E.1. The values for the repeatability limit and reproducibility limit have been derived from the results of interlaboratory tests carried out in accordance with ISO 5725-1<sup>[4]</sup> and ISO 5725-2<sup>[5]</sup>.

Table E.1 — Animal feeding stuffs

Parameter	Swine diet	Cattle feed	Swine finish	Pig feed	Swine grower	Chick feed	Corn soya bean mix	Turkey meal	Dog food	Protein concentrate	Mixed feeding stuff	Poultry diet	Beef finisher, high NPN
No. laboratories	9	10	9	10	9	9	9	10	10	10	30	9	9
No. results	9	20	9	20	9	18	9	20	20	20	150	9	9
Mean total nitrogen content % mass fraction	1,74	1,87	2,15	2,43	2,51	3,22	3,26	4,18	4,64	6,73	9,05	9,8	13,1
Standard deviation of repeatability, $s_r$ %	0,023	0,021	0,022	0,023	0,029	0,027	0,041	0,035	0,030	0,022	0,73	0,43	0,37
Coefficient of variation of repeatability, $CV(r)$ %	1,296	1,103	1,025	0,927	1,139	0,830	1,256	0,846	0,638	0,333	8,07	4,39	2,82
Repeatability limit, $r (= 2,8 s_r)$	0,063	0,058	0,062	0,064	0,080	0,076	0,115	0,100	0,084	0,063	2,07	1,22	1,05
Standard deviation of reproducibility, $s_R$ %	0,058	0,039	0,051	0,040	0,066	0,022	0,057	0,043	0,036	0,049	0,97	0,90	0,15
Coefficient of variation of reproducibility, $CV(R)$ %	3,311	2,083	2,357	1,652	2,628	0,694	1,734	1,019	0,765	0,732	10,72	9,18	1,15
Reproducibility limit, $R (= 2,8 s_R)$	0,161	0,111	0,142	0,114	0,184	0,063	0,158	0,120	0,100	0,140	2,75	2,55	0,43
Reference	[18]	a	[18]	a	[18]	a	[18]	a	a	a	[28]	[18]	[18]

a The collaborative study was carried out at the national level in 1991 by AFNOR.

Table E.2 — Animal feeding stuffs — Feed meals

Parameter	Meat and bone meal	Fish meal	Gluten meal	Meat meal	Feather meal	Blood meal
No. laboratories	9	30	10	10	9	9
No. results	9	150	20	20	9	9
Mean total nitrogen content % mass fraction	8,6	9,2	10,38	13,55	13,67	14,07
Standard deviation of repeatability, $s_r$ %	0,38	0,088	0,046	0,050	0,047	0,026
Coefficient of variation of repeatability, $CV(r)$ %	4,42	0,914	0,444	0,370	0,340	0,186
Repeatability limit, $r$ (= 2,8 $s_r$ )	1,08	0,246	0,130	0,142	0,130	0,073
Standard deviation of reproducibility, $s_R$ %	0,75	0,332	0,100	0,116	0,080	0,112
Coefficient of variation of reproducibility, $CV(R)$	8,72	3,609	0,958	0,856	0,583	0,798
Reproducibility limit, $R$ (= 2,8 $s_R$ )	2,13	0,930	0,282	0,328	0,223	0,314
Reference	[18]	a	a	a	[18]	[28]

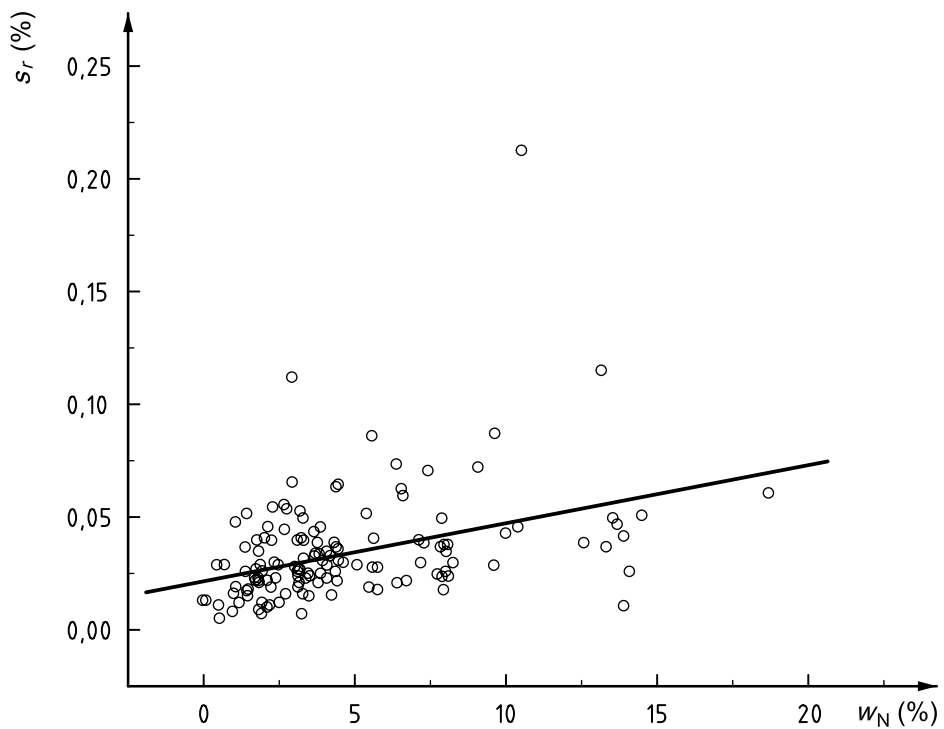
<sup>a</sup> The collaborative study was carried out at the national level in 1991 by AFNOR.



Table E.4 — Oilseed meals

Parameter	Safflower meal	Safflower meal	Rape meal	Cottonseed meal	Soya bean meal	Canola meal	Soya bean meal	Soya bean meal	Soya bean meal	Soya bean meal	Peanut meal
No. laboratories	23	24	10	24	24	23	9	10	24	24	24
No. results	45	47	20	92	47	45	9	20	91	91	91
Mean total nitrogen content % mass fraction	3,32	3,34	5,41	6,62	7,13	7,21	7,30	7,42	7,88	7,88	8,25
Standard deviation of repeatability, $s_r$ %	0,050	0,040	0,052	0,060	0,040	0,030	0,039	0,071	0,050	0,050	0,030
Coefficient of variation of repeatability, $CV(r)$ %	1,506	1,198	0,961	0,906	0,561	0,416	0,540	0,953	0,635	0,635	0,364
Repeatability limit, $r$ (= 2,8 $s_r$ )	0,140	0,112	0,147	0,168	0,112	0,084	0,110	0,200	0,140	0,140	0,084
Standard deviation of reproducibility, $s_R$ %	0,060	0,110	0,070	0,070	0,080	0,040	0,075	0,088	0,060	0,060	0,070
Coefficient of variation of reproducibility, $CV(R)$ %	1,807	3,293	1,297	1,057	1,122	0,555	1,031	1,182	0,761	0,761	0,848
Reproducibility limit, $R$ (= 2,8 $s_R$ )	0,168	0,308	0,199	0,196	0,224	0,112	0,211	0,248	0,168	0,168	0,196
Reference	[18]	[18]	a	[18]	[18]	[18]	[18]	a	[18]	[18]	[18]

<sup>a</sup> The collaborative study was carried out at the national level in 1991 by AFNOR.



**Key**

$s_r$  standard deviation of repeatability

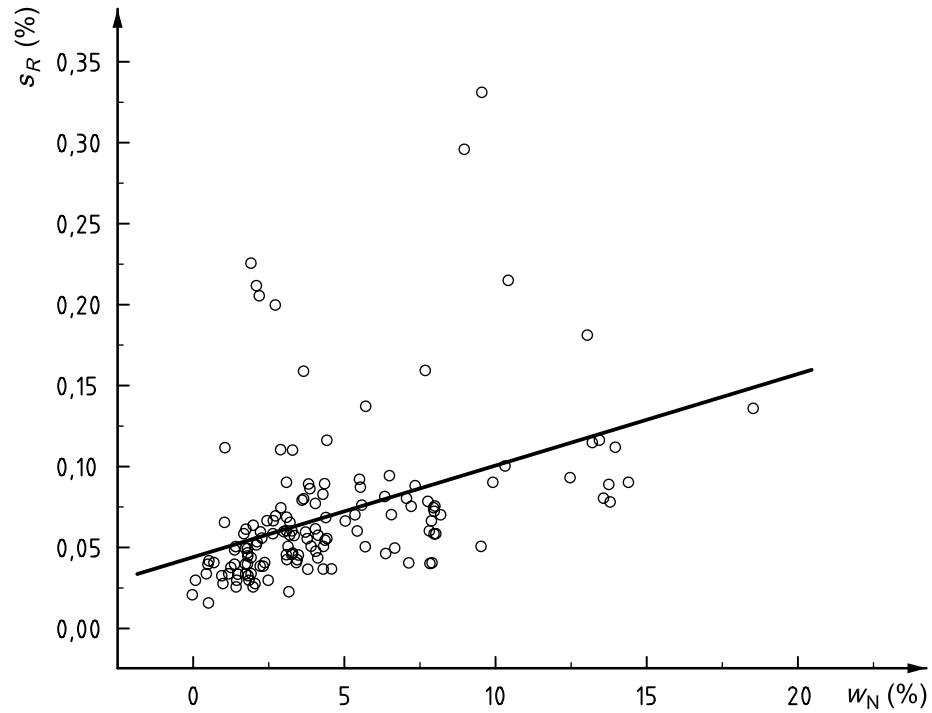
$w_N$  nitrogen mass fraction

$$s_r = 0,022 + 0,0026w_N$$

correlation coefficient:  $R^2 = 0,380$

**Figure E.1 — Relationship between standard deviation of repeatability and nitrogen percentage mass fraction for determinations by the Dumas method (compilation of data in this annex)**





**Key**

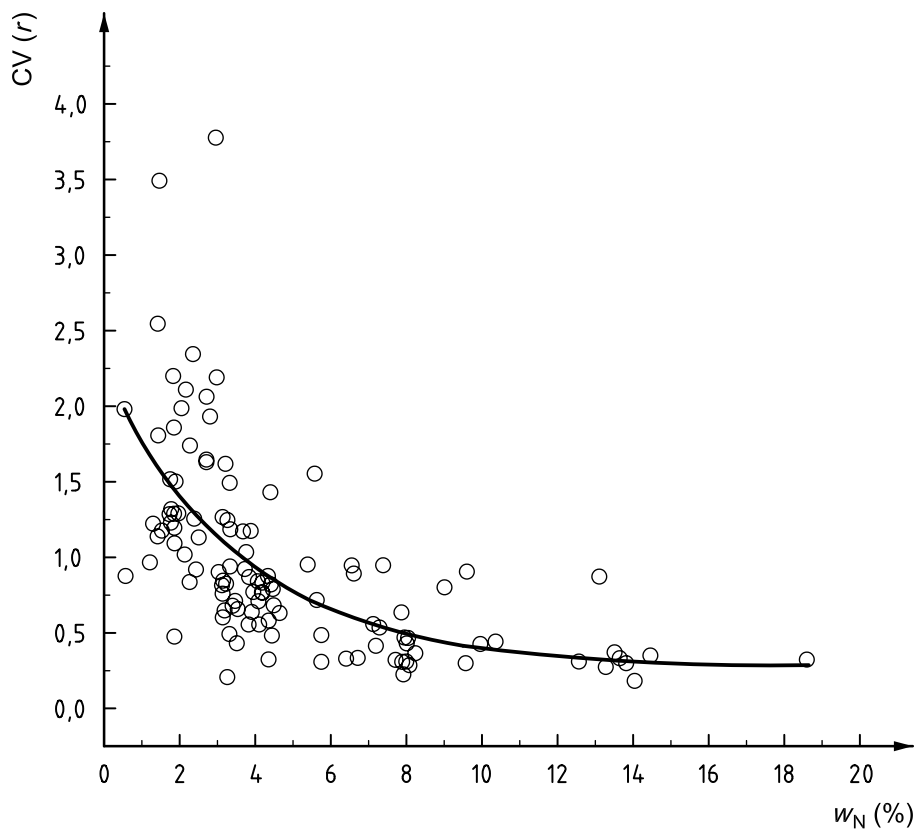
$s_R$  standard deviation of reproducibility

$w_N$  nitrogen mass fraction

$$s_R = 0,044 + 0,0057w_N$$

correlation coefficient:  $R^2 = 0,409$

**Figure E.2 — Relationship between standard deviation of reproducibility and nitrogen percentage mass fraction for determinations by the Dumas method (compilation of data in this annex)**

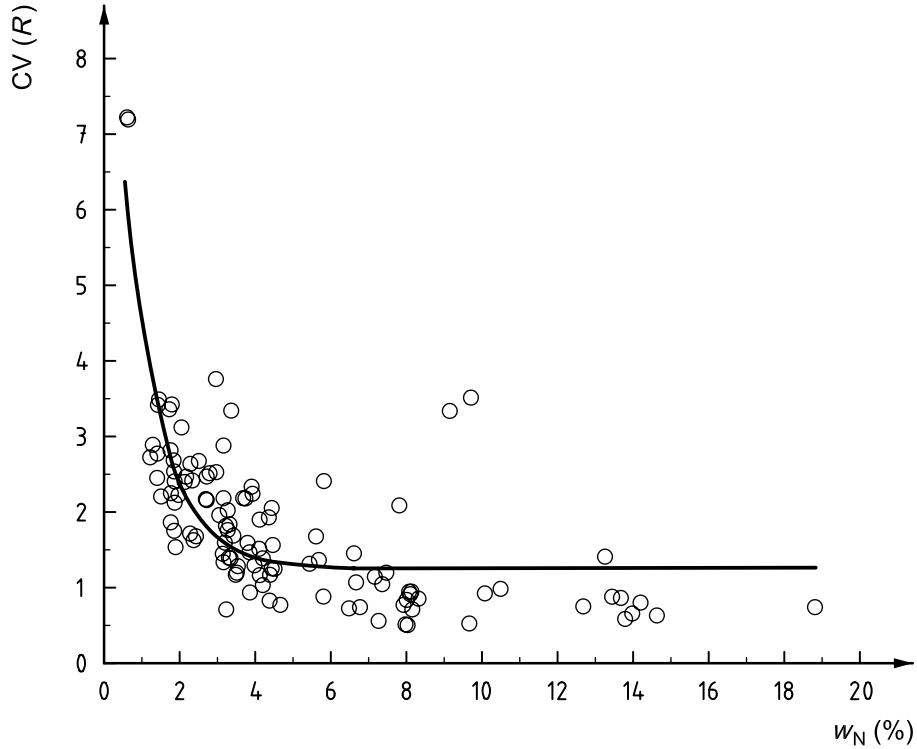


**Key**

$CV(r)$  coefficient of variation of repeatability

$w_N$  nitrogen mass fraction

**Figure E.3 — Relationship between coefficient of variation of repeatability and nitrogen percentage mass fraction for determinations by the Dumas method (compilation of data in this annex)**



**Key**

CV(R) coefficient of variation of reproducibility  
w<sub>N</sub> nitrogen mass fraction

**Figure E.4 — Relationship between coefficient of variation of reproducibility and nitrogen percentage mass fraction for determinations by the Dumas method (compilation of data in this annex)**

**Annex F**  
(informative)

**Relationship between Dumas nitrogen and Kjeldahl nitrogen**

Table F.1 — Nitrogen mass fractions estimated by the Dumas and Kjeldahl methods

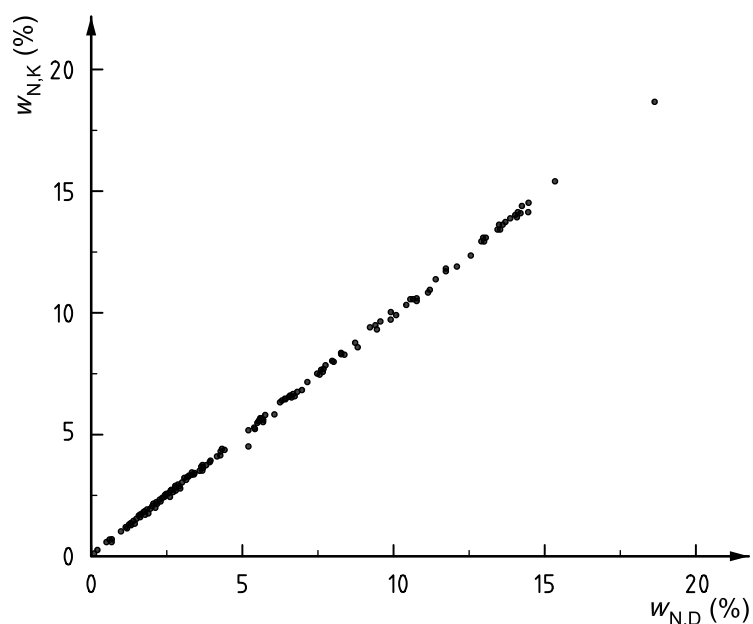
Commodity	Kjeldahl details	Dumas instrument used <sup>a</sup>	Range of nitrogen	Ratio between the means for Kjeldahl/Dumas protein	Notes	Conversion equation <sup>b</sup>	References
Soybean products	Micro-Kjeldahl: copper-potassium sulfate	Elementar RapidN III	0,08 to 14,1	0,66 to 1,03		$w_{N,K} = 0,974 8w_{N,D} - 0,004 9$	[39]
Animal feeds and raw materials	Kjel-Foss	Foss-Heraeus MacroN	0,7 to 12,9	0,93 to 1,02	Compared to Kjel-Foss	$w_{N,K} = 0,993 9w_{N,D} - 0,043$	[33]
Feed samples	Copper(II) sulfate	LECO CNS 2000	0,13 to 10,2			$w_{N,K} = 0,998 7w_{N,D} - 0,056 7$	[26]
Dairy products and standards	Copper(II) sulfate	LECO and Foss-Heraeus	0,5 to 14,5	0,979 to 1,029		$w_{N,K} = 0,994 6w_{N,D} + 0,024 8$	[43]
Animal feeds and raw materials	Copper(II) sulfate	LECO FP228	2,6 to 14	0,927 to 1,000	High-nitrate orchard grass	$w_{N,K} = 1,002w_{N,D} - 0,055 9$	[42]
Animal feeds and raw materials	Mercury catalyst	LECO FP428/CHN 600	1,6 to 15	0,975 to 1,010	Nicotinic acid ratio, 0,871 3	$w_{N,K} = 0,999 8w_{N,D} + 0,013 4$	[42]
Grains and oilseeds	Mercury catalyst	LECO Perkin Elmer Heraeus	1,92 to 5,6	0,97 to 1,010	Nicotinic acid ratio, 0,95	$w_{N,K} = 1,000 5w_{N,D} - 0,017 3$	[21]
Barley and malt	Various catalysts including mercury(II) oxide	LECO and Foss-Heraeus	1,145 to 2,03	0,978 to 0,997	All Kjeldahl	$w_{N,K} = 0,967 9w_{N,D} + 0,013 8$	[22]
					Less Kjel-Foss	$w_{N,K} = 0,965 7w_{N,D} + 0,030 6$	
Wheat and wheat flour					Bias about 0,2 % protein		[36]
Cereals, oilseeds, grains		Non-commercial			Instrument finds 1,2 % to 2,5 % more protein in oilseeds but similar for oilseeds	Instrument finds 1,2 % to 2,5 % more protein in oilseeds but similar for oilseeds	[31]
Yeast, spent grains malt					Instrument finds 0,1 % to 0,4 % more protein, not a significant difference statistically	Instrument finds 0,1 % to 0,4 % more protein, not a significant difference statistically	[26]
Oilseeds (canola, flax soybeans, sunflower seed, mustard)	Titanium oxide optimized to wheat	LECO FP228	3,7 to 7,5		Differences of 0,15 % in $w_N$ also sunflower seed noted as a problem	$w_{N,K} = 0,973 7w_{N,D} + 0,021 3$	[23]

Table F.1 (continued)

Commodity	Kjeldahl details	Dumas instrument used <sup>a</sup>	Range of nitrogen	Ratio between the means for Kjeldahl/Dumas protein	Notes	Conversion equation <sup>b</sup>	References
<b>Wide range of materials</b>	Copper(II) sulfate	LECO FP228	0,07 to 37,4	0,33 to 1,40	Noted variance in fruits and vegetables and high variance in fish	$w_{N,K} = 1,00w_{N,D} - 0,09$	[41]
<b>Feeding stuffs</b>	Kjel-Foss	LECO FP228	0,5 to 14	small: 0,98 to 1,84; macro: 0,93 to 1,87	Compared the small sample (100 mg) in AOAC method to a 1 g pressed sample. The large sample method had much better repeatability, nicotinic acid ratio, 0,98, but EDTA at 0,83; possible error in paper	small: $w_{N,K} = 0,997w_{N,D} - 0,086$ large: $w_{N,K} = 0,988w_{N,D} - 0,014$	[40]
<b>Baby foods</b>	Copper(II) sulfate	CE Model 1500	1,01 to 9,26	0,95 to 1,01		$w_{N,K} = 0,979w_{N,D} - 0,003$	[38]

<sup>a</sup> This information is given for the convenience of the users of this International Standard and does not constitute an endorsement by ISO of these products.

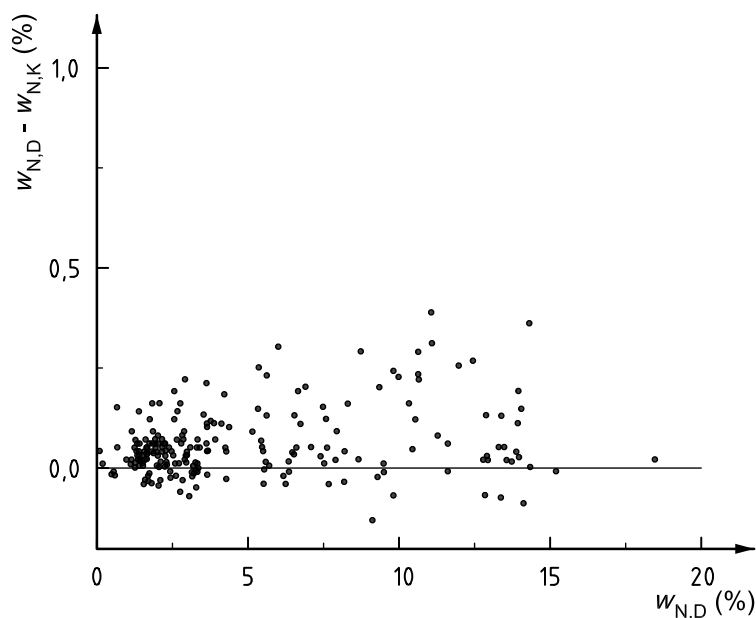
<sup>b</sup>  $w_{N,K}$  is the Kjeldahl nitrogen content, expressed as a percentage mass fraction;  $w_{N,D}$  is the Dumas nitrogen content, expressed as a percentage mass fraction.



**Key**

- $w_{N,D}$  nitrogen mass fraction determined by the Dumas method
- $w_{N,K}$  nitrogen mass fraction determined by the Kjeldahl method

**Figure F.1 — Relationship between nitrogen mass fractions estimated by the Dumas and Kjeldahl methods for samples from studies reported in Table F.1**



**Key**

- $w_{N,D} - w_{N,K}$  difference between nitrogen mass fraction determined by the Dumas and Kjeldahl methods
- $w_{N,D}$  nitrogen mass fraction determined by the Dumas method
- NBS standard orchard leaves
- $w_{N,D} = 5,1$  % mass fraction;  $w_{N,K} = 4,5$  % mass fraction; expected value of  $w_N = 5,0$  % mass fraction

**Figure F.2 — Residual plot for relationship between nitrogen mass fractions estimated by the Dumas and Kjeldahl methods for samples from studies reported in Table F.1**

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DMRC Building, Bhavbhuti Marg, New  
Delhi 110002

Telephones

{ 2323 7617

Eastern : 8<sup>th</sup> Floor, Plot No 7/7 & 7/8, CP Block, Sector V,  
Salt Lake, Kolkata, West Bengal 700091

{ 2367 0012  
2320 9474

Northern : Plot No. 4-A, Sector 27-B, Madhya Marg,  
Chandigarh 160019

{ 265 9930

Southern : C.I.T. Campus, IV Cross Road, Taramani, Chennai 600113

{ 2254 1442  
2254 1216

Western : 5<sup>th</sup> Floor/MTNL CETTM Technology Street, Hiranandani Gardens, Powai,  
Mumbai - 400076

{ 25700030  
25702715

**Branches :** AHMEDABAD, BENGALURU, BHOPAL, BHUBANESHWAR, CHANDIGARH, CHENNAI, COIMBATORE, DEHRADUN, DELHI, FARIDABAD, GHAZIABAD, GUWAHATI, HARYANA (CHANDIGARH), HUBLI, HYDERABAD, JAIPUR, JAMMU, JAMSHEDPUR, KOCHI, KOLKATA, LUCKNOW, MADURAI, MUMBAI, NAGPUR, NOIDA, PARWANOO, PATNA, PUNE, RAIPUR, RAJKOT, SURAT, VIJAYAWADA.