
**Cereals and pulses — Determination
of the nitrogen content and calculation
of the crude protein content —
Kjeldahl method**

*Céréales et légumineuses — Détermination de la teneur en azote et
calcul de la teneur en protéines brutes — Méthode de Kjeldahl*



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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. 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.

The committee responsible for this document is ISO/TC 34, *food and food products*, Subcommittee SC 4, *cereals and pulses*.

This second edition cancels and replaces the first edition (ISO 20483:2006), which has been technically revised.

Cereals and pulses — Determination of the nitrogen content and calculation of the crude protein content — Kjeldahl method

WARNING — The use of this International Standard can involve hazardous materials, operations and equipment. This International Standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish appropriate safety practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This International Standard specifies a method for the determination of the nitrogen content of cereals, pulses and derived products, according to the Kjeldahl method, and a method for calculating the crude protein content.

The method does not distinguish between protein nitrogen and non-protein nitrogen. If it is important to determine the non-protein nitrogen content, an appropriate method would be applied.

NOTE In certain cases, full recovery of the nitrogen in nitrates and nitrites is not possible by this method.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable to 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

quantity of nitrogen determined after application of the procedure described

Note 1 to entry: It is expressed as a mass fraction of dry product, as a percentage.

3.2

crude protein content

quantity of crude protein obtained from the nitrogen content as determined by applying the specified method, calculated by multiplying this content by an appropriate factor depending on the type of cereal or pulse

Note 1 to entry: It is expressed as a mass fraction of dry product, as a percentage.

4 Principle

A test portion is digested by sulfuric acid in the presence of a catalyst. The reaction products are made alkaline, then distilled. The liberated ammonia is collected in a boric acid solution, which is titrated with a sulfuric acid solution, in order to determine the nitrogen content and calculate the crude protein content.

5 Reagents

WARNING — The reagents described in [5.3](#), [5.8](#), [5.9](#) and [5.13](#) shall be handled with caution.

5.1 Use only nitrogen-free reagents of recognized analytical grade, except for the reference materials, and distilled or demineralized water or water of equivalent purity

5.2 Kjeldahl tablets, corresponding to the following composition: copper (II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) = 2,8 %, titanium oxide (TiO_2) = 2,8 % and potassium sulfate (K_2SO_4) = 94,3 %.

Alternatively, copper(II) sulfate pentahydrate, titanium oxide and potassium sulfate may also be mixed in the corresponding ratio.

5.3 Sulfuric acid, $c(\text{H}_2\text{SO}_4) = 18 \text{ mol/l}$, $\rho_{20}(\text{H}_2\text{SO}_4) = 1,84 \text{ g/ml}$.

5.4 Antifoaming agent: Paraffin oil, silicone or even antifoam tablets may be used to prevent foaming.

5.5 Acetanilide ($\text{C}_8\text{H}_9\text{NO}$) or tryptophan ($\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_2$), of minimum assay 99 % (mass fraction).

5.6 Boric acid, aqueous solution, $\rho_{20}(\text{H}_3\text{BO}_3) = 40 \text{ g/l}$, or any other concentration recommended for the apparatus being used.

5.7 Coloured indicator

Add volumes of Solution A ([5.7.1](#)) and Solution B ([5.7.2](#)) as recommended for the apparatus being used (for example: 5 volumes of Solution A and 1 volume of Solution B) or any other coloured indicator recommended for the apparatus.

NOTE 1 It is possible to use a ready-to-use solution of boric acid containing the coloured indicator ([5.7.1](#) and [5.7.2](#)).

NOTE 2 The ratio of Solutions A and B can be adjusted depending on the apparatus.

The titration may also be carried out potentiometrically by the use of a pH electrode, which shall be checked every day.

5.7.1 Solution A

Bromocresol green ($\text{C}_{21}\text{H}_{14}\text{Br}_4\text{O}_5\text{S}$): 200 mg.

Ethanol ($\text{C}_2\text{H}_5\text{OH}$), with a volume fraction of 95 %: quantity sufficient for 100 ml of solution.

5.7.2 Solution B

Methyl red ($\text{C}_{15}\text{H}_{15}\text{N}_3\text{O}_2$): 200 mg.

Ethanol ($\text{C}_2\text{H}_5\text{OH}$), with a volume fraction of 95 %: quantity sufficient for 100 ml of solution.

5.8 Sodium hydroxide, aqueous solution (NaOH), having a mass fraction of between 30 % and 40 %, with nitrogen content less than or equal to 0,001 %.

Technical grade sodium hydroxide may also be used when its nitrogen content is less than or equal to 0,001 %.

5.9 Sulfuric acid, standard volumetric solution, $c(\text{H}_2\text{SO}_4) = 0,05 \text{ mol/l}$.

The use of H_2SO_4 instead of HCl is recommended because H_2SO_4 does not have the tendency to produce bubbles in the connecting tubes.

5.10 Ammonium sulfate, standard volumetric solution, $c(\text{NH}_4)_2\text{SO}_4 = 0,05 \text{ mol/l}$.

Alternatively, a salt such as $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ may be used.

5.11 Pumice stone, granulated, washed in hydrochloric acid and ignited or glass boiling rods may be used to prevent bumping.

5.12 Sucrose (optional), free from nitrogen.

5.13 Diphosphorus pentoxide (P_2O_5).

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Mechanical grinder.

6.2 Sieve, with aperture size 0,8 mm.

6.3 Analytical balance, capable of weighing to the nearest 0,001 g.

6.4 Digestion, distillation and titration apparatus.

The homogeneous temperature distribution of the digestion unit should be ascertained.

The homogeneity of the temperature should be ensured out by carrying out a full test with one of the two reference materials (5.5), and considering the recovery rates obtained.

The distillation apparatus should also be verified by conducting the distillation of a known quantity of ammonium salt [e.g. 10 ml of an ammonium sulfate solution (5.10)], and by checking that the recovery rate is greater than or equal to 99,8 %.

7 Sampling

Sampling is not part of the method specified in this International Standard. Recommended sampling methods are given in ISO 24333.

It is important the laboratory receive a truly representative sample which has not been damaged or changed during transport or storage.

8 Preparation of test sample

If necessary, grind the sample so that it passes entirely through a sieve with 0,8 mm aperture size. For grains, a mass of at least 200 g should be ground. Mix the ground sample thoroughly.

9 Determination of the moisture content

Determine the moisture content, w_H , of the test sample from an aliquot of the sample prepared according to [Clause 8](#). Carry out the determination by following the method adapted to the product under test (i.e. ISO 712 for cereals and cereal products, ISO 6540 for maize, the method described in Reference [\[8\]](#) and which is used for testing certain pulses, or ISO 24557 for pulses).

10 Procedure

10.1 General

If it is required to check that the requirements given concerning the repeatability limit ([12.2](#)) are fulfilled, carry out two separate determinations in accordance with [10.2](#) to [10.5](#).

10.2 Test portion

Weigh, to the nearest 0,001 g, a mass of test sample prepared according to [Clause 8](#), chosen on the basis of the assumed nitrogen content, so that the test portion contains between 0,005 g and 0,2 g of nitrogen and preferably more than 0,02 g.

10.3 Determination

10.3.1 Digestion

WARNING — The following operations should be conducted under a well-ventilated, sulfuric acid-resistant hood.

Transfer the test portion ([10.2](#)) to the digestion flask. Then, add the required number of catalyst tablets ([5.2](#)), containing 10 g of potassium sulfate, 0,30 g of copper(II) sulfate pentahydrate and 0,30 g of titanium oxide. In the end, add 20 ml of sulfuric acid ([5.3](#)).

The quantity of acid may be adjusted depending on the apparatus, but only after having made certain that this measure indeed leads to a recovery rate of 99,5 % for acetanilide and 99,0 % for tryptophan.

Carefully mix so as to ensure a thorough wetting of the test portion.

Place the flasks in the digestion unit preheated to (420 ± 10) °C.

After a minimum of 2 h of digestion, counted from the time the unit temperature reached (420 ± 10) °C again, leave to cool.

NOTE It is advisable to add pumice stone or glass boiling rods ([5.11](#)) as a boiling regulator and an antifoaming agent ([5.4](#)).

The minimum digestion time shall be checked on that reference material with which it was most difficult to reach the recovery rate (see [10.5](#)).

Follow the recommendations of the equipment manufacturer as far as evacuation of the vapours is concerned, because too strong a suction can result in a loss of nitrogen.

10.3.2 Distillation

Cautiously add to the cooled flask 50 ml of water and leave to cool. Transfer into the collecting flask 50 ml of boric acid ([5.6](#)) and, for visual colourimetry or using an optical probe, at least 10 drops of coloured indicator ([5.7](#)).

Add an **excess** of 5 ml of the sodium hydroxide solution ([5.8](#)) required for neutralizing the quantity of sulfuric acid used. Then carry out the distillation.

Depending on the equipment, the quantities of reagents used may vary.

10.3.3 Titration

Carry out the titration using the sulfuric acid solution (5.9), either continuously during the distillation or at the end of distillation on all of the distillate.

The end-point determination is judged by visual colourimetry, or using an optical probe, or by potentiometric analysis with a pH measurement system.

10.4 Blank test

Perform a blank test with the reagents used in 10.3.1 to 10.3.3 but without the test sample (10.2).

NOTE It is possible to replace the test sample with 1 g of sucrose (5.12).

10.5 Test with reference material (check test)

Dry the reference material(s) used at a temperature of between 60 °C and 80 °C, under vacuum, in the presence of *di*-phosphorus pentoxide (5.13).

Carry out a check test on a test portion of a minimum of 0,15 g by determining the nitrogen content of the tryptophan and/or of the acetanilide (5.5).

NOTE It is possible to add 1 g of sucrose (5.12) to reference material.

The nitrogen recovery rate from acetanilide shall be at least 99,5 % and at least 99,0 % from tryptophan.

11 Expression of results

11.1 Nitrogen content

The nitrogen content, w_N , expressed as a mass fraction of dry product, as a percentage (%), is obtained using Formula (1):

$$w_N = \frac{(V_1 - V_0) \times T \times 0,014 \times 100}{m} \times \frac{100}{100 - w_H} = \frac{140 T (V_1 - V_0)}{m(100 - w_H)} \quad (1)$$

where

V_0 is the volume, in millilitres, of the sulfuric acid solution (5.9) required for the blank test;

V_1 is the volume, in millilitres, of the sulfuric acid solution (5.9) required for the test portion;

0,014 is the value, in grams, of the quantity of nitrogen equivalent to the use of 1 ml of a 0,5 mol/l sulfuric acid solution;

T is the normality of the sulfuric acid solution used for the titration;

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

w_H is the moisture content, determined according to [Clause 9](#).

Express the result to two decimal places.

11.2 Crude protein content

Calculate the crude protein content of the dry product by multiplying the value obtained at the time of determination of the nitrogen content (11.1) by a conversion factor adapted to the types of cereal or pulse and to their use.

Express the result to one decimal place.

NOTE Some conversion factors used for cereals are given in Annex C. For others, 6,25 is generally used.

12 Precision

12.1 Interlaboratory test

Details of interlaboratory tests on the precision of the method are summarized in Annex A. The values derived from these interlaboratory tests might not be applicable to concentration ranges and matrices other than those given.

12.2 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 the repeatability limit, r , calculated from Formula (2):

$$r = (0,0063 \times w_p) \times 2,8 \quad (2)$$

where w_p is the crude protein content of the sample expressed as a mass fraction of dry product, as a percentage (see Table B.1).

For products whose crude protein content is between 7 % and 80 %, see Table A.1 and Figure A.1.

12.3 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 the reproducibility limit, R , calculated from Formula (3):

$$R = (0,014 \times w_p) \times 2,8 \quad (3)$$

(See Table B.1.)

For products whose crude protein content is between 7 % and 80 %, see Table A.1 and Figure A.1.

12.4 Critical difference

12.4.1 Comparison of two groups of measurements in one laboratory

The critical difference (CD_r) between two averages, each obtained from two test results under repeatability conditions, is equal to:

$$CD_r = 1,98 \times s_r = 1,98 \times (0,0063 \times w_p) = 0,01247 \times w_p \quad (4)$$

(See Table B.1.)

where

s_r is the repeatability standard deviation;

w_p is the crude protein content of the sample expressed as a mass fraction of dry product, as a percentage.

12.4.2 Comparison of two groups of measurements in two laboratories

The critical difference (CDR) between two averages, each obtained from two test results by two different laboratories under repeatability conditions, is equal to:

$$\text{CDR} = 2,8\sqrt{s_R^2 - 0,5s_r^2} = 2,8\sqrt{(0,014 \times w_p)^2 - 0,5 \times (0,0063 \times w_p)^2} = 0,03716 \times w_p \quad (5)$$

(See [Table B.1](#).)

where

s_R is the reproducibility standard deviation;

s_r is the repeatability standard deviation;

w_p is the crude protein content of the sample expressed as a mass fraction of dry product, as a percentage.

13 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, i.e. ISO 20483;
- d) the conversion factor used (see Note to [11.2](#));
- e) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which might have influenced the test result(s);
- f) the test result(s) obtained or, if the repeatability has been checked, the final quoted result obtained.

Annex A (informative)

Results of interlaboratory tests

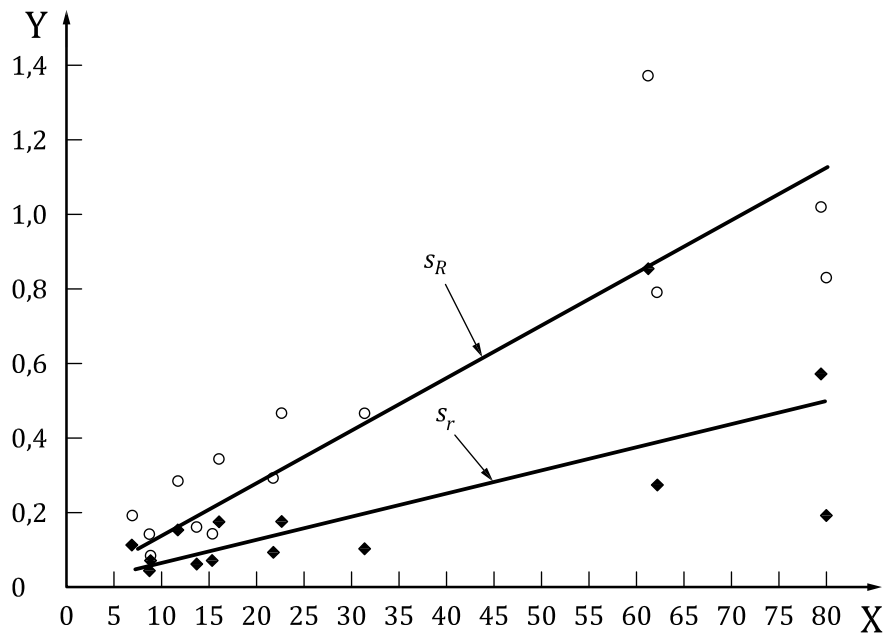
The repeatability, reproducibility and critical difference of the method were established by two interlaboratory tests circuits conducted in accordance with the requirements of ISO 5725-2, ISO 5725-3 and ISO 5725-6.

Ten laboratories took part in this test. Fourteen products and four reference materials were analysed. The results are given in [Table A.1](#).

Table A.1 — Statistical results of the interlaboratory tests

Parameter(s)	Sample ^a													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Number of laboratories retained after elimination of the outliers	10	9	10	10	10	10	10	10	10	10	10	10	10	9
Mean protein content ($w_N \times 5,7$), % of dry matter, w_P	7,03	8,94	9,02	11,88	13,90	15,54	16,19	21,91	22,80	31,57	61,33	62,19	79,46	79,99
Standard deviation of repeatability, s_r	0,11	0,04	0,07	0,15	0,06	0,07	0,17	0,09	0,17	0,10	0,85	0,27	0,57	0,19
Coefficient of variation (standard deviation r /mean), %	1,56	0,45	0,78	1,26	0,43	0,45	1,05	0,41	0,75	0,32	1,39	0,43	0,72	0,24
Repeatability limit, $r (= 2,8 \times s_r)$	0,31	0,11	0,20	0,42	0,17	0,20	0,48	0,25	0,48	0,28	2,38	0,76	1,60	0,53
Standard deviation of reproducibility, s_R	0,19	0,14	0,08	0,28	0,16	0,14	0,34	0,29	0,46	0,46	1,37	0,79	1,02	0,83
Coefficient of variation (standard deviation R /mean), %	2,70	1,57	0,89	2,36	1,15	0,90	2,10	1,32	2,02	1,46	2,23	1,27	1,28	1,04
Reproducibility limit, $R (= 2,8 s_R)$	0,53	0,39	0,22	0,78	0,45	0,39	0,95	0,81	1,29	1,29	3,84	2,21	2,86	2,32

^a Samples were: 1 = Common wheat flour 1; 2 = Maize; 3 = Barley; 4 = Common wheat 1; 5 = Common wheat flour 3; 6 = Durum wheat; 7 = Common wheat flour 2; 8 = Peas 2; 9 = Peas 1; 10 = Field beans; 11 = Wheat gluten 1; 12 = Wheat gluten 2; 13 = Maize gluten 1; 14 = Maize gluten 2

**Key**

- X protein content, %
 Y standard deviation, %
 s_R reproducibility standard deviation, (o)
 s_r repeatability standard deviation, (♦)

Figure A.1 — Relationship between standard deviations of repeatability and reproducibility and the mean value of the protein content

[Figure A.1](#) shows that the standard deviations of repeatability and reproducibility depend on the crude protein values found and are therefore not constant.

The establishment of the functional relationship between the precision (repeatability or reproducibility) values and the mean level of proteins gave rise to several types of relationships.

The minor differences observed between these relationships led to adopting the equation passing through zero:

- a) equation of the regression line for s_r $s_r = 0,0063 \times w_P$
 b) determination coefficient $R^2 = 0,4646$
 c) equation of the regression line for s_R $s_R = 0,014 \times w_P$
 d) determination coefficient $R^2 = 0,7849$

Annex B (informative)

Critical difference and practical application of the repeatability and reproducibility limits to different protein contents

B.1 Comparison of two groups of measurements in one laboratory

The critical difference (CDr) between two averaged values obtained from two test results under repeatability conditions is equal to:

$$CDr = 2,8 s_r \sqrt{\frac{1}{2n_1} + \frac{1}{2n_2}} \quad (B.1)$$

where

s_r is the standard deviation of repeatability;

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

NOTE Critical difference is the difference between two averaged values obtained from two test results under repeatability conditions; see ISO 5725-6.

If n_1 and n_2 are both equal to 2, the equation reduces to:

$$CDr = 2,8 s_r \sqrt{\frac{1}{2}} = 1,98 s_r \quad (B.2)$$

B.2 Comparison of two groups of measurements in two laboratories

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

$$CDR = 2,8 \sqrt{s_R^2 - s_r^2} \left(1 - \frac{1}{2n_1} - \frac{1}{2n_2} \right) \quad (B.3)$$

where

s_r is the standard deviation of repeatability;

s_R is the standard deviation of reproducibility;

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

If n_1 and n_2 are both equal to 2, the equation reduces to:

$$CDR = 2,8 \sqrt{s_R^2 - 0,5s_r^2} \quad (B.4)$$

Table B.1 — Practical application of the repeatability and reproducibility limits to different protein contents

Protein content ($w_N \times 5,7$) %	Standard deviation of repeatability s_r	Repeatability limit r	Standard deviation of reproducibility s_R	Reproducibility limit R	Critical difference (CD) between two means	
					in one laboratory	between two laboratories
10	0,06	0,17	0,14	0,39	0,12	0,37
15	0,09	0,25	0,21	0,59	0,18	0,56
20	0,12	0,34	0,28	0,78	0,24	0,75
25	0,15	0,42	0,35	0,98	0,30	0,93
30	0,18	0,50	0,42	1,18	0,36	1,12
35	0,21	0,59	0,49	1,37	0,42	1,31
40	0,24	0,67	0,56	1,57	0,48	1,49
45	0,27	0,76	0,63	1,76	0,53	1,68
50	0,30	0,84	0,70	1,96	0,59	1,87
55	0,33	0,92	0,77	2,16	0,65	2,05
60	0,36	1,01	0,84	2,35	0,71	2,24
65	0,39	1,09	0,91	2,55	0,77	2,43
70	0,42	1,18	0,98	2,74	0,83	2,61
75	0,45	1,26	1,05	2,94	0,89	2,80
80	0,48	1,34	1,12	3,14	0,95	2,99

Let:

$$\boxed{\text{Test 1}} + \boxed{\text{Test 2}} = \boxed{\text{Mean 1 (test 1 + test 2) / 2}}$$

$$\boxed{\text{Test 5}} + \boxed{\text{Test 6}} = \boxed{\text{Mean 2 (test 5 + test 6) / 2}}$$

$$\boxed{\text{Test 9}} + \boxed{\text{Test 10}} = \boxed{\text{Mean 3 (test 9 + test 10) / 2}}$$

EXAMPLE

The repeatability limit is applied between

or between

The reproducibility limit is applied between

or between

The critical difference (CD) is applied between

or between

test 1 and test 2

test 5 and test 6

test 1 and test 6

test 2 and test 9

mean 1 and mean 2

mean 1 and mean 3

Annex C (informative)

Factors for converting nitrogen content to protein content

Table C.1

Commodity	Nitrogen to protein conversion factor
Common wheat	5,7
Durum wheat	5,7
Wheat milling products	5,7 or 6,25
Wheat for feed	6,25
Barley	6,25
Oats	5,7 or 6,25
Rye	5,7
Triticale	6,25
Corn	6,25
Pulses	6,25

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- [3] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*
- [4] ISO 5725-3, *Accuracy (trueness and precision) of measurement methods and results — Part 3: Intermediate measures of the precision of a standard measurement method*
- [5] ISO 5725-6, *Accuracy (trueness and precision) of measurement methods and results — Part 6: Use in practice of accuracy values*
- [6] ISO 5983-1, *Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content — Part 1: Kjeldahl method*
- [7] ISO 24333, *Cereals and cereal products — Sampling*
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