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Glavni naslov: Določitev vsebnosti dušika in izračun vsebnosti surovega proteina v žitaricah in stročnicah - Kjeldahlova metoda (ISO 20483:2006)

Cereals and pulses - Determination of the nitrogen content and calculation of the crude protein content - Kjeldahl method (ISO 20483:2006)

Getreide und Hülsenfrüchte - Bestimmung des Stickstoffgehaltes und Berechnung des Rohproteingehaltes - Kjeldahl-Verfahren (ISO 20483:2006)

Céréales et légumineuses - Détermination de la teneur en azote et calcul de la teneur en protéines brutes - Méthode de Kjeldahl (ISO 20483:2006)

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**Ta slovenski standard je istoveten z: EN ISO 20483:2006**

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**ICS:**

67.060 žitarice in stročnice in izdelki iz njih Cereals, pulses and derived products

**SIST EN ISO 20483:2007**

**en**

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ICS 67.060

English Version

Cereals and pulses - Determination of the nitrogen content and calculation of the crude protein content - Kjeldahl method (ISO 20483:2006)

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EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

## **Foreword**

This document (EN ISO 20483:2006) has been prepared by Technical Committee ISO/TC 34 "Agricultural 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of an identical text or by endorsement, at the latest by May 2007, and conflicting national standards shall be withdrawn at the latest by May 2007.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

### **Endorsement notice**

The text of ISO 20483:2006 has been approved by CEN as EN ISO 20483:2006 without any modifications.

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**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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Published in Switzerland

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## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 20483 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

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

**WARNING** — The use of this standard may involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this 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 can be applied.

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

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## 2 Normative references

SIST EN ISO 20483:2007

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

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

## 3 Terms and definitions

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

### 3.1

#### **nitrogen content**

quantity of nitrogen determined after application of the procedure described in this International Standard

NOTE It is expressed as mass fraction of dry product, in percent.

### 3.2

#### **crude protein content**

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

NOTE It is expressed as mass fraction of dry product, in percent.

## 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, to determine the nitrogen content and calculate the crude protein content.

## 5 Reagents

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

**WARNING — The reagents described in 5.4, 5.8, 5.11 and 5.12 shall be handled with precautions.**

**5.1 Potassium sulfate** ( $K_2SO_4$ ).

**5.2 Copper(II) sulfate pentahydrate** ( $CuSO_4 \cdot 5H_2O$ ).

**5.3 Titanium oxide** ( $TiO_2$ ).

**5.4 Sulfuric acid**,  $c(H_2SO_4) = 18 \text{ mol/l}$ ,  $\rho_{20}(H_2SO_4) = 1,84 \text{ g/ml}$ .

**5.5 Paraffin oil.**

**5.6 Acetanilide** ( $C_8H_9NO$ ), having a melting point of  $114^\circ C$  and nitrogen content of  $10,36 \text{ g/100 g}$ .

**5.7 Tryptophan** ( $C_{11}H_{12}N_2O_2$ ), having a melting point of  $282^\circ C$  and nitrogen content of  $13,72 \text{ g/100 g}$ .

**5.8 Phosphorus pentoxide** ( $P_2O_5$ ).

**5.9 Boric acid**, aqueous solution,  $\rho_{20}(H_3BO_3) = 40 \text{ g/l}$ , or any other concentration recommended for the apparatus being used.

**5.10 Coloured indicator**

Add volumes of Solution A (5.10.1) and Solution B (5.10.2) as recommended for the apparatus being used (for example: 5 volumes of Solution A and 1 volume of Solution B).

NOTE 1 It is possible to use a ready-to-use solution of boric acid containing the coloured indicator (5.9 + 5.10).

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

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

### 5.10.1 Solution A

Bromocresol green ( $C_{21}H_{14}Br_4O_5S$ ): 200 mg.

Ethanol ( $C_2H_5OH$ ), with a volume fraction of 95 %: quantity sufficient for 100 ml solution.

### 5.10.2 Solution B

Methyl red ( $C_{15}H_{15}N_3O_2$ ): 200 mg.

Ethanol ( $C_2H_5OH$ ), with a volume fraction of 95 %: quantity sufficient for 100 ml solution.

**5.11 Sodium hydroxide**, aqueous solution (NaOH), with a mass fraction of 33 %, or a mass fraction of 40 %, with nitrogen content less than or equal to 0,001 %.

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

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

The use of  $\text{H}_2\text{SO}_4$  instead of HCl is recommended because  $\text{H}_2\text{SO}_4$  does not have the tendency to produce bubbles in the connecting tubes.

**5.13 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.

**5.14 Pumice stone**, granulated, washed in hydrochloric acid and ignited.

**5.15 Sucrose** (optional), free from nitrogen.

## 6 Apparatus

**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 assessment of temperature homogeneity should be done by carrying out a whole test with one of the two reference materials (5.6 or 5.7) 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 (5.13)] and by checking that the recovery rate is greater than or equal to 99,8 %.

## 7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. Recommended sampling methods are given in ISO 6644 and ISO 13690.

## 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.