SLOVENSKI STANDARD
SIST EN ISO 13903:2005

01-september-2005

Krma - Določ evanje amino kislin (ISO 13903:2005)

Animal feeding stuffs - Determination of amino acids content (ISO 13903:2005)

Futtermittel - Bestimmung des Aminosäuregehalts (ISO 13903:2005)

Aliments des animaux - Détermination de la teneur en acides aminés (ISO 13903:2005)

Ta slovenski standard je istoveten z: EN ISO 13903:2005

ICS:

65.120	Krmila	Animal feeding stuffs

SIST EN ISO 13903:2005 **en**

EUROPEAN STANDARD

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English version

Animal feeding stuffs - Determination of amino acids content (ISO 13903:2005)

Aliments des animaux - Détermination de la teneur en acides aminés (ISO 13903:2005)

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This European Standard was approved by CEN on 19 April 2005.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

This document (EN ISO 13903:2005) has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" in collaboration with Technical Committee CEN/TC 327 "Animal feeding stuffs - Methods of sampling and analysis", the secretariat of which is held by NEN.

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of an identical text or by endorsement, at the latest by November 2005, and conflicting national standards shall be withdrawn at the latest by November 2005.

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, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

Endorsement notice

The text of ISO 13903:2005 has been approved by CEN as EN ISO 13903:2005 without any modifications.

INTERNATIONAL STANDARD

ISO 13903

First edition
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Animal feeding stuffs — Determination of amino acids content

Aliments des animaux — Détermination de la teneur en acides aminés

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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 13903 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*.

ISO 13903 is based on Commission Directive 98/64/EC of September 1998 [1].

Animal feeding stuffs — Determination of amino acids content

1 Scope

This International Standard describes the determination of free (synthetic and natural) and total (peptide-bound and free) amino acids in feeding stuffs, using an amino acid analyser or HPLC equipment. It is applicable to the following amino acids:

— sum of cystine and cysteine;
— methionine;
— lysine;
— threonine;
— alanine;
— arginine;
— aspartic acid;
— glutamic acid;
— glycine;
— histidine;
— isoleucine;
— leucine;
— phenylalanine;
— proline;
— serine;
— tyrosine;
— valine.

The method does not distinguish between the salts of amino acids, nor does it differentiate between D and L forms of amino acids. It is not valid for the determination of tryptophan or hydroxy analogues of amino acids.

Limits of quantification depend on the chromatographic equipment, but levels as low as: 0,3 g/kg total lysine; 0,25 g/kg total methionine; 0,35 g/kg total cystine plus cysteine; 0,2 g/kg total threonine; 0,035 g/kg free lysine; 0,035 g/kg free methionine; and 0,03 g/kg free threonine can typically be analysed.

NOTE A lower limit of quantification or detection might be achievable but this is to be validated by the users.

2 Principle

2.1 Free amino acids

The free amino acids are extracted with dilute hydrochloric acid. Co-extracted nitrogenous macromolecules are precipitated with sulfosalicylic acid and removed by filtration. The filtered solution is adjusted to pH 2,20. The amino acids are separated by ion exchange chromatography and determined by reaction with ninhydrin with photometric detection at 570 nm.

2.2 Total amino acids

The procedure chosen depends on the amino acids under investigation. Cyst(e)ine and methionine shall be oxidized to cysteic acid and methionine sulphone, respectively, prior to hydrolysis. Tyrosine shall be determined in hydrolysates of unoxidized samples. All the other amino acids listed in Clause 1 may be determined in either the oxidized or unoxidized sample.

Oxidation is performed at 0 °C with a performic acid/phenol mixture. Excess oxidation reagent is decomposed with sodium disulfite. The oxidized or unoxidized sample is hydrolysed with hydrochloric acid (c = 6 mol/l) for 23 h. The hydrolysate is adjusted to pH 2,20. The amino acids are separated by ion exchange chromatography and determined by reaction with ninhydrin, using photometric detection at 570 nm (440 nm for proline).

3 Reagents and materials

Use only reagents of recognized analytical grade, unless otherwise specified.

3.1 Water, double distilled water or water of equivalent quality shall be used (conductivity < 10 μS).

3.2 Hydrogen peroxide, w = 30 %.

3.3 Formic acid, w = 98 % to 100 %.

3.4 Hydrochloric acid, density approximately 1,19 g/ml.

3.5 2,2'-Thiodiethanol (thiodiglycol)

3.6 Light petroleum, boiling rate 40 °C to 60 °C

3.7 Norleucine, or any other compound suitable for use as internal standard.

3.8 Nitrogen gas (< 10 parts per million oxygen).

3.9 Amino acids.

3.9.1 Standard substances listed under Clause 1.

Use pure compounds containing no water of crystallization. Dry under vacuum over P_2O_5 or H_2SO_4 for 1 week prior to use.

3.9.2 Cysteic acid.

3.9.3 Methionine sulfone.

3.10 Sodium hydroxide solution I, c = 7,5 mol/l.

Dissolve 300 g of NaOH (3.6) in water and make up to 1 l.

3.11 Sodium hydroxide solution II, c = 1 mol/l.

Dissolve 40 g of NaOH in water (3.1) and make up to 1 l.

3.12 Formic acid-phenol solution.

Mix 889 g of formic acid (3.3) with 111 g of water (3.1) and add 4,73 g of phenol.

3.13 Hydrolysis mixture, c = 6 mol/l HCl containing 1 g of phenol per litre.

Add 1 g of phenol to 492 ml of HCl (3.4) and make up to 1 l with water (3.1).

3.14 Extraction mixture, c = 0,1 mol/l HCl containing 2 % thiodiglycol.

Take 8,2 ml of HCl (3.4), dilute with approximately 900 ml of water (3.1). Add 20 ml of thiodiglycol (3.5) and make up to 1 l with water. Do not mix 3.4 and 3.5 directly.

3.15 5-Sulfosalicylic acid, β = 6 %.

Dissolve 60 g of 5-sulfosalicylic acid dihydrate in water (3.1) and make up to 1 l with water.

3.16 Oxidation mixture (performic acid-phenol).

Mix 0,5 ml of hydrogen peroxide (3.2) with 4,5 ml of formic acid-phenol solution (3.12) in a small beaker. Incubate at between 20 °C and 30 °C for 1 h in order to form performic acid, then cool in an ice-water bath (15 min) before adding to the sample.

Avoid contact with skin and wear protective clothing.

3.17 Citrate buffer, c = 0,2 mol/l Na^+, pH 2,20.

Dissolve 19,61 g of sodium citrate dihydrate, 5 ml of thiodiglycol (3.5), 1 g of phenol and 16,50 ml of HCl (3.4) in approximately 800 ml of water (3.1). Adjust the pH to 2,20. Make up to 1 l with water.

3.18 Elution buffers, prepared according to conditions for the analyser used (4.9).

3.19 Ninhydrin reagent, prepared according to conditions for the analyser used (4.9).

3.20 Standard solutions of amino acids

These solutions shall be stored below 5 °C.

3.20.1 Stock standard solution of amino acids (3.9.1), c = 2,5 μmol/ml of each in hydrochloric acid.

These may be obtained commercially.

3.20.2 Stock standard solution of cysteic acid and methionine sulfone, c = 1,25 μmol/ml.

Dissolve 0,211 5 g of cysteic acid (3.9.2) and 0,226 5 g of methionine sulphone (3.9.3) in citrate buffer (3.17) in a 1 l graduated flask and make up to mark with citrate buffer. Store below 5 °C for not more than 12 months. This solution shall not be used if the stock standard solution (3.20.1) contains cysteic acid and methionine sulfone.

3.20.3 Stock standard solution of the internal standard e.g. norleucine, c = 20 μmol/ml.

Dissolve 0,656 0 g of norleucine (3.7) in citrate buffer (3.17) in a graduated flask and make up to 250 ml with citrate buffer. Store below 5 °C for no more than 6 months.

3.20.4 Calibration solution of standard amino acids, for use with hydrolysates, c = 0,1 μmol/ml of cysteic acid and methionine sulfone and c = 0,2 μmol/ml of the other amino acids.

Dissolve 2,2 g of sodium chloride in 100 ml beaker with 30 ml of citrate buffer (3.17). Add 4,00 ml of stock standard solution of amino acids (3.20.1), 4,00 ml of stock standard solution of cysteic acid and methionine sulfone (3.20.2), and 0,50 ml of stock standard solution of internal standard (3.20.3) if used. Adjust the pH to 2,20 with sodium hydroxide (3.11). Transfer quantitatively to a 50 ml graduated flask and make up to the mark with citrate buffer (3.17) and mix. Store below 5 °C for not more than 3 months. See also 9.1.