SLOVENSKI STANDARD
SIST EN ISO 15914:2005

01-september-2005

Krma - Encimsko določ evanje celotnega ġkroba (ISO 15914:2004)

Animal feeding stuffs - Enzymatic determination of total starch content (ISO 15914:2004)

Futtermittel - Enzymatische Bestimmung von Stärke (ISO 15914:2004)

Aliments des animaux - Détermination enzymatique de la teneur totale en amidon (ISO 15914:2004)

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65.120	Krmila	Animal feeding stuffs

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Animal feeding stuffs - Enzymatic determination of total starch content (ISO 15914:2004)

Aliments des animaux - Détermination enzymatique de la teneur totale en amidon (ISO 15914:2004)

Futtermittel - Enzymatische Bestimmung von Stärke (ISO 15914:2004)

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Foreword

The text of ISO 15914:2004 has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 15914:2005 by 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of an identical text or by endorsement, at the latest by September 2005, and conflicting national standards shall be withdrawn at the latest by September 2005.

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Endorsement notice

The text of ISO 15914:2004 has been approved by CEN as EN ISO 15914:2005 without any modifications.

INTERNATIONAL STANDARD

ISO 15914

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Animal feeding stuffs — Enzymatic determination of total starch content

Aliments des animaux — Détermination enzymatique de la teneur totale en amidon

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ISO 15914:2004(E)

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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 15914 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*.

Animal feeding stuffs — Enzymatic determination of total starch content

1 Scope

This international Standard specifies a method for the enzymatic determination of the total starch content of animal feeding stuffs and raw materials for animal feeding stuffs.

The method is also applicable to the determination of the purity of starch.

It is important that in the sample matrix no components are present which contribute to the measured extinction at 340 nm.

The analytical range of the method is 40 g/kg to 1 000 g/kg starch. The standard procedure is applicable to the range 200 g/kg to 1000 g/kg. For the lower range, 40 g/kg to 200 g/kg, another dilution procedure for the standard glucose solution and samples can be used.

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 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 6498:1998, *Animal feeding stuffs — Preparation of test samples*

3 Terms and definitions

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

3.1
starch
natural vegetable polymer consisting of long linear unbranched chains of 1,4-α-D-glucose units (amylose) and/or long α-1,6-branched chains of α-1,4-linked glucose units (amylopectin)

3.2
starch content
mass fraction of starch and its high molecular mass breakdown products, insoluble in 40 % ethanol, and determined in accordance with the method of this International Standard

NOTE The starch content is expressed in grams per kilogram.

4 Principle

The milled test sample is extracted with 40 % ethanol to remove the soluble sugars. Sample disintegration and starch solubilization is achieved by dispersing the extracted test portion in aqueous DMSO (90 % volume fraction) at 100 °C, followed by addition of concentrated hydrochloric acid at 60 °C. The solubilized and dissolved starch is quantitatively converted into glucose by the enzyme amyloglucosidase. The glucose quantification is carried out by the well-known hexokinase method (see [1], [2]).

5 Reagents

Use only reagents of recognized analytical grade.

5.1 Water, complying with at least grade 3 in accordance with ISO 3696:1987.

5.2 Ethanol (C_2H_5OH), 40 % (volume fraction)

Take 417 ml of ethanol (96 % volume fraction) and dilute with water to 1 000 ml

5.3 Hydrochloric acid, c(HCl) = 12 mol/l.

5.4 Aqueous sodium hydroxide, c(NaOH) = 4 mol/l.

Dissolve in a beaker 40 g of NaOH in about 50 ml water. After cooling, transfer quantitatively to a 250 ml volumetric flask and dilute to the mark with water.

WARNING — Heat develops. Wear safety glasses.

5.5 Acetic acid solution, $c(CH_3COOH)$ = 2 mol/l.

Add to a 500 ml volumetric flask about 200 ml water, followed by 59 ml of glacial acetic acid. Dilute to the mark with water.

5.6 Sodium acetate solution, $c(CH_3COONa)$ = 2 mol/l.

Dissolve, in a 500 ml volumetric flask, 82,0 g of sodium acetate in water and dilute to the mark with water.

5.7 Sodium acetate buffer, $c(CH_3COONa/H)$ = 2 mol/l, pH = 4,8.

Mix 41 ml of acetic acid solution (5.5) with 59 ml of sodium acetate solution (5.6). Check the pH with a pH-meter and, if necessary, adjust to obtain the correct pH with the acetic acid or the sodium acetate solution.

Prepare a fresh buffer solution daily.

5.8 Aqueous dimethylsulfoxide, (σ_{DMSO}) = 90 % (volume fraction).

Mix pure DMSO and water in a volume ratio of 9:1.

5.9 Clarifying solutions, according to Carrez, prepared as follows.

5.9.1 Potassium hexacyanoferrate(II) solution, $c[K_4Fe(CN)_6]$ = 0,25 mol/l.

In a 1 litre volumetric flask, dissolve 106 g of potassium hexacyanoferrate(II) trihydrate [$K_4Fe(CN)_6 \cdot 3H_2O$] in water. Dilute to the mark with water.

5.9.2 Zinc acetate, solution in 0,5 mol/l acetic acid, $c[Zn(CH_3CO_2)_2]$ = 1 mol/l.

In a 1 litre volumetric flask, dissolve 219,5 g of zinc acetate dihydrate [$Zn(CH_3CO_2)_2{\cdot}2H_2O$] and 30 g of glacial acetic acid in water. Dilute to the mark with water.

5.10 Iodine solution in potassium iodide

In a 1 litre volumetric flask, dissolve 12,7 g of iodine (I_2) and 24,0 g of potassium iodide (KI) in water. Dilute to the mark with water. Dilute this solution 10-fold before use.

5.11 Standard glucose solution

5.11.1 Samples containing 200 g/kg to 1 000 g/kg starch

Prepare three independent standard glucose solutions (c = 0,0194 mol/l). In each 100 ml volumetric flask dissolve 350 mg anhydrous glucose ($C_6H_{12}O_6$) to the nearest mg in water. Dilute to the mark with water.

5.11.2 Samples containing 40 g/kg to 200 g/kg starch

Prepare three independent standard glucose solutions (c = 0,0039 mol/l). In each 500 ml volumetric flask, dissolve 350 mg ± 1 mg of anhydrous glucose ($C_6H_{12}O_6$) in water. Dilute to the mark with water.

Prepare fresh standard glucose solutions daily.

5.12 Amyloglucosidase solution, 160 U/ml AMG.

Dissolve, in a mixture of 9 ml of water and 1 ml of sodium acetate buffer (5.7), 267 mg of amyloglucosidase (AMG) [EC 3.2.1.3 (*Aspergillus niger*, Roche Diagnostics, No. 1 202 367, 6 U/mg)].[1)] With respect to storage and the use of the enzymes, follow carefully the recommendations of the enzyme supplier.

When another enzyme is used, the activity should be estimated as given in Note 1. The mass of the enzyme should be adapted to the activity found.

NOTE 1 Different enzyme suppliers use different definitions for the units of activities of enzymes. In this International Standard the following definition for the unit of activity for AMG has been used: 1 unit of amyloglucosidase will release 1 µmol of glucose from glycogen in 1 min at 25 °C and pH = 4,75.

NOTE 2 10 ml of enzyme solutions is enough for about 75 determinations.

5.13 D-Glucose UV test set, for quantifying glucose enzymaticly, with the hexokinase method (R-Biopharm, No. 716251[2)], according to the manufacturer, the unused kits may be stored for 1 year at 4 °C), as given in 5.13.1 to 5.13.3.

5.13.1 Buffer/substrate solution (Bottle 1)

Dissolve the content of Bottle 1 in 45 ml of freshly prepared distilled water. Store this reagent in a cool (4 °C) and dark place for not longer than for 4 weeks. Use the required amount of this solution at ambient temperature.

5.13.2 Enzyme solution (Bottle 2)

This solution is ready for use.

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1) Roche Diagnostics No. 1 202 367 is an example of a suitable product 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 product.

2) R-Biopharm AG No. 716251 is an example of a suitable product 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 products.