FINAL DRAFT
International Standard

ISO/FDIS 15914-2

Animal feeding stuffs — Enzymatic determination of total starch content —

Part 2: Method by enzymatic determination with a hexokinase system and potassium hydroxide dispersion

Alimentation animale — Détermination enzymatique de la teneur totale en amidon —
Partie 2: Méthode par dosage enzymatique avec un système hexokinase et dispersion à l'hydroxyde de potassium

ISO/TC **34**/SC **10**

Secretariat: **INSO**

Voting begins on:
2024-03-05

Voting terminates on:
2024-04-30

Reference number
ISO/FDIS 15914-2:2024(en)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

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 document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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

A list of all parts in the ISO 15914 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Animal feeding stuffs — Enzymatic determination of total starch content —

Part 2: Method by enzymatic determination with a hexokinase system and potassium hydroxide dispersion

1 Scope

This document specifies an enzymatic method for determining starch in animal feeding stuffs containing starchy ingredients (cereals, tubers, etc.). The method is also applicable to beans and to the animal digestive contents because it involves the hexokinase system for the final glucose determination.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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, *Water for analytical laboratory use — Specification and test methods*

ISO 6498, *Animal feeding stuffs — Guidelines for sample preparation*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Principle

Washing of the sample with a volume fraction of 40 % ethanol to eliminate the soluble sugars and soluble starch degradation products. Dispersion of the residue by means of potassium hydroxide, hydrolysis of starch into glucose units with amyloglucosidase, determination of the glucose obtained with a hexokinase system.

5 Reagents

Unless otherwise specified, use only reagents of recognized analytical grade and distilled water or water of equivalent purity.

NOTE Reagents 5.7 to 5.10 are also marketed in the form of a ready-to-use kits.

5.1 Water, conforming to at least grade 3 in accordance with ISO 3696.

5.2 40 % ethanol (volume fraction) prepared as follows: Pour 400 ml of absolute ethanol into a 1 l volumetric flask and make up the volume with water.

5.3 Potassium hydroxide solution, at 1 mol/l.

5.4 Acetic acid, minimum purity of 96 %.

5.5 Amyloglucosidase of Aspergillus niger, glucose-free. The activity shall be checked when opening a new batch using the method described in Annex A.

5.6 Aqueous solution of amyloglucosidase (5.5) with an activity of (1 500 ± 100) units/ml and prepared extemporaneously.

A unit is defined as being a µmole of glucose released/min/gram of enzyme.

5.7 Triethanolamine buffer solution, prepared as follows:

In a 250 ml beaker, weigh 14 g of triethanolamine hydrochloride and 0,25 g of magnesium sulfate heptahydrate, add 80 ml of water and dissolve. Then, add 5 ml of 5 N aqueous soda, homogenize and bring to pH 7,6 ± 0,1 using the potassium hydroxide solution (5.3). Transfer to a 100 ml volumetric flask and make up the volume with water. Agitate and keep in the refrigerator.

5.8 NADPH solution, prepared as follows:

Dissolve 60 mg of NADPH disodium salt in 6 ml of water. This solution can be kept in the refrigerator for at least four weeks.

5.9 ATP solution, prepared as follows:

In 6 ml of water, dissolve 300 mg of sodium bicarbonate and 300 mg of ATP disodium salt. This solution can be kept in the refrigerator for four weeks.

5.10 Suspension of HK/G6P-DH, prepared as follows:

Mix 1 ml of ammonium sulfate solution (3,2 mol/l), 280 U of hexokinase (EC 2.7.1.1) and 140 U of glucose-6-phosphate dehydrogenase (EC 1.1.1.49). This solution can be kept in the refrigerator for at least one year.

6 Equipment

The usual laboratory equipment and, in particular, the following shall be used.

6.1 Thermostatic bath, with magnetic or mechanical agitation, set to (60 ± 2) °C.

6.2 Centrifuge tubes, of 100 ml made of glass.

6.3 Centrifuge, for centrifuging the tubes (6.2) at about 2,000*g*.

6.4 Grinder, suitable for final crushing to a particle size of ≤ 0,5 mm. The percentage of particles passing through a 0,5 mm screen shall be 95 % or more.

6.5 Spectrophotometer, ultraviolet (UV)/visible set to 340 nm or 365 nm.

6.6 pH-meter, for measurement to within 0,1 units pH.

6.7 Ultrasonic tank.