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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

FDIS stage

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

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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 ~~describes~~specifies an enzymatic method for determining starch in animal feeding stuffs containing starchy ingredients (cereals, tubers, etc.). The method is also ~~applies~~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 6498, *Animal feeding stuffs — Guidelines for sample preparation*~~

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 ~~(v/v)~~ 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, ~~complying with~~conforming to at least grade 3 in accordance with ISO 3696.

5.2 40 % ethanol (~~v/v~~volume fraction) prepared as follows: Pour 400 ml of absolute ethanol into a ~~one litre~~1 l volumetric flask and make up ~~to~~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~~the 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.

~~NOTE The reagents (5.7) to (5.10) are also marketed in the form of a ready-to-use kits.~~

6 Equipment

~~Standard~~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~~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.

6.8 Micropipettes, for sampling volumes of 0,020 ml and 0,100 ml, verified beforehand.

6.9 Analytical balance, allowing weighing to the nearest 1 mg.