INTERNATIONAL STANDARD

ISO 6493

First edition
2000-02-01

Animal feeding stuffs — Determination of starch content — Polarimetric method

Aliments des animaux — Détermination de la teneur en amidon — Méthode polarimétrique

Reference number
ISO 6493:2000(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 734 10 79
E-mail copyright@iso.ch
Web www.iso.ch

Printed in Switzerland

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 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 6493 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 10, *Animal feeding stuffs*.

Annexes A and B of this International Standard are for information only.

Animal feeding stuffs — Determination of starch content — Polarimetric method

1 Scope

This International Standard specifies a method for the polarimetric determination of the starch content of animal feeding stuffs and raw materials for animal feeding stuffs.

The method is not applicable to products which, besides starch, contain other substances which are optically active during the analysis and do not dissolve in 40 % ethanol. Examples of these products are potato pulp, beet chips, beet leaves, beet tops, yeast, soya products, lupine and products rich in inulin (e.g. chicory roots and Jerusalem artichokes). In these cases an enzymatic determination of the starch content may be used.

This method is not applicable for the quantification of starch with an amylose content exceeding 40 % (e.g. high amylose maize starch like Hylon VII).

CAUTION — Depending on the intensity of the heat/moisture treatment the product has been subjected to, too low a starch content may be determined.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 3310-1, *Test sieves — Technical requirements and testing — Part 1: Test sieves of metal wire cloth*.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*.

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

3 Terms and definitions

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

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

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

NOTE The starch content is expressed in grams per kilogram.

4 Principle

A test portion is decomposed with dilute hydrochloric acid, then the solubilized starch is gelatinized and partially hydrolysed.

The total optical rotation of the clarified solution is determined.

Correction is made for the optical rotation caused by other substances which are soluble in 40 % ethanol and optically active after treatment with dilute hydrochloric acid.

The starch content is calculated by multiplying the corrected optical rotation by a known factor.

5 Reagents

Use only reagents of recognized analytical grade.

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

5.2 Ethanol (C_2H_5OH), 40 % volume fraction.

5.3 Methyl red, solution in ethanol (96 % volume fraction), ρ(methyl red) = 1 g/l.

5.4 Hydrochloric acid, c(HCl) = 0,31 mol/l.

Check the concentration by titration with a 0,100 mol/l sodium hydroxide solution using methyl red as indicator. An aliquot portion of 10 ml of hydrochloric acid shall neutralize (31,0 ± 0,1) ml of the sodium hydroxide solution.

CAUTION — Too high or too low a hydrochloric acid concentration will lead to incorrect values of the determined starch content.

5.5 Hydrochloric acid, c(HCl) = 7,73 mol/l.

5.6 Clarifying solutions, according to Carrez, as follows.

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

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

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

In a 1 l 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.

5.7 Saccharose solution, $\rho(C_{12}H_{22}O_{11})$ = 100,0 g/l.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Analytical balance, capable of weighing to the nearest 1 mg.

6.2 pH-meter, capable of measuring to the nearest 0,1 pH unit.

6.3 Boiling water bath, with sufficient capacity to maintain the water at boiling point while immersing the conical flasks.

CAUTION — If the water bath is not boiling constantly, too high a starch content will be determined.

6.4 Polarimeter, accurate to at least 0,01° and suitable for 200 mm long tubes.

Measure the optical rotation at a wavelength of 589,3 nm (sodium D line). When using polarimeter tubes of deviating lengths, measure with a corresponding accuracy.

A saccharimeter may be used if it has an accuracy of measurement at least equal to the accuracy of the polarimeter. In this case, convert the readings to degrees.

The polarimeter may be calibrated with the saccharose solution (5.7). This saccharose solution yields an optical rotation of 13,30° when measured at (20 ± 1) °C using a 200 mm polarimeter tube.

6.5 Burette.

6.6 Reflux condensers.

6.7 Volumetric flasks, of capacity 100 ml.

If the volumetric flasks need to be fitted to a reflux condenser (see 9.3.3), the use of wide-necked Kohlrausch conical flasks is recommended.

NOTE Kohlrausch conical flasks are commercially available volumetric flasks used for sugar determinations.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497 [1].

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

8 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

If solid, grind the laboratory sample (usually 500 g) so that it passes completely through a sieve with 0,5 mm apertures complying with ISO 3310-1. Mix thoroughly.

9 Procedure

9.1 Determination of acid consumption

9.1.1 Weigh approximately 2,5 g of the prepared test sample to the nearest 1 mg and transfer it quantitatively to a 50 ml conical flask. Add 25 ml of water and shake until a homogeneous suspension is obtained.

9.1.2 Place the electrodes of the pH-meter (6.2) in the suspension and, using a burette, add hydrochloric acid (5.4) until the pH of the suspension is $3,0 \pm 0,1$. Check whether the complete acid consumption of the test portion has been compensated for, by shaking the suspension vigorously and allowing it to stand for 2 min. If during this period the pH has increased up to a value exceeding 3,1, add more hydrochloric acid (5.4), using the burette, as many times as necessary until no more acid is consumed.

9.1.3 Calculate the acid consumption of the test portion from the volume of hydrochloric acid (5.4) added.

9.2 Determination of total optical rotation

9.2.1 Weigh approximately 2,5 g of the prepared test sample (m_1) to the nearest 1 mg and transfer it quantitatively to a dry 100 ml volumetric flask (6.7). Add 25 ml of hydrochloric acid (5.4). Shake until a homogeneous suspension is obtained, then add a further 25 ml of hydrochloric acid (5.4).

9.2.2 Compensate for the acid consumption of the test portion (see 9.1) by adding hydrochloric acid of a suitable concentration so that the volume of the contents of the flask is changed by not more than 1 ml.

EXAMPLE Suppose that 5,0 ml of 0,1 mol/l hydrochloric acid is used in 9.1.2 for compensation of a sample rich in chalk, then the acid consumption of the test portion is 0,5 mmol (9.1.3). In that case, add 0,5 ml of 1,0 mol/l hydrochloric acid in 9.2.2.

CAUTION — If the hydrochloric acid concentration in the suspension deviates from 0,31 mol/l, a wrong starch content will be determined. Too high or too low a hydrochloric acid concentration will result in too low or too high values, respectively, of the determined starch content.

9.2.3 Immerse the flask in the boiling water bath (6.3). During the first 3 min, regularly shake the flask vigorously to prevent clotting and to obtain equal heat distribution in the suspension. When shaking, keep the flask immersed.

In the case of multiple analyses, immerse the flasks over suitable time intervals so as to keep the water bath at boiling point.

After 15 min $\pm$ 5 s, remove the conical flask from the bath. Immediately add 30 ml of water (5.1) at a temperature not exceeding 10 °C and swirl. Cool to a temperature of about 20 °C in a water bath with running cold water.

CAUTION — If the flask stays in the boiling water bath too long or if the temperature decrease is too slow, too low a starch content will be determined.

Add 5 ml of potassium hexacyanoiron(II) solution (5.6.1) and shake for 1 min. Add 5 ml of zinc acetate solution (5.6.2) and shake again for 1 min. Dilute to the mark with water, mix and filter. Discard the first few millilitres of the filtrate.

Determine the optical rotation of the filtrate (α_1) with the polarimeter or saccharimeter (6.4).

9.3 Determination of optical rotation of ethanol-soluble substances

9.3.1 Weigh approximately 5 g of the prepared test sample (m_2) to the nearest 1 mg and transfer it quantitatively to a dry 100 ml volumetric flask (6.7). Add 40 ml of ethanol (5.2). Shake until a homogeneous suspension is obtained then add a further 40 ml of ethanol (5.2).

9.3.2 Compensate for the acid consumption of the test portion (see 9.1) by adding hydrochloric acid of a suitable concentration so that the volume of the contents of the flask is changed by not more than 1 ml. In principle, the amount of hydrochloric acid to be added is twice as much as that added in 9.2.2.

9.3.3 Shake vigorously and leave to stand for 1 h at room temperature. During this time, shake vigorously at least every 10 min.

If the lactose content of the sample exceeds 50 g/kg (as for whey powder and separated milk powder), dissolve the sample by heating the flask, fitted with a reflux condenser, in a water bath at (50 ± 2) °C for 30 min.

Dilute to the mark with ethanol (5.2), mix and filter. Discard the first few millilitres of the filtrate.

Pipette 50 ml of the filtrate into a 100 ml volumetric flask (6.7). Add 2,0 ml of hydrochloric acid (5.5) and swirl vigorously. Fit a reflux condenser to the flask and immerse the flask in the boiling water bath (6.3).

After 15 min ± 5 s, remove the conical flask from the bath. Immediately add 30 ml of water (5.1) at a temperature not exceeding 10 °C and swirl. Cool to a temperature of about 20 °C in a water bath with running cold water.

Add 5 ml of potassium hexacyanoiron(II) solution (5.6.1) and shake for 1 min. Add 5 ml of zinc acetate solution (5.6.2) and shake again for 1 min. Dilute to the mark with water, homogenize and filter. Discard the first few millilitres of the filtrate.

Determine the optical rotation of the clear filtrate (α_2) with the polarimeter or saccharimeter (6.4).

10 Calculation and expression of results

Calculate the starch content of the test sample by the equation:

$$w = \frac{20\,000}{\alpha_D^{20}} \times \left[\frac{2{,}5\alpha_1}{m_1} - \frac{5\alpha_2}{m_2} \right]$$

where

w is the numerical value of the starch content of the test sample, in grams per kilogram;

α_1 is the numerical value of the total optical rotation, in degrees, measured in 9.2;

α_2 is the numerical value of the optical rotation, in degrees, of the ethanol-soluble substances measured in 9.3;

m_1 is the numerical value of the mass, in grams, of the test portion for the determination of the total optical rotation (9.2);

m_2 is the numerical value of the mass, in grams, of the test portion for the determination of the optical rotation of the ethanol-soluble substances (9.3);

α_D^{20} is the numerical value of the specific optical rotation, in degrees, of pure starch measured at a wavelength of 589,3 nm (Na D line):

α_D^{20} = 185,9 for rice starch;

α_D^{20} = 185,7 for potato starch (see annex A);

α_D^{20} = 184,6 for maize starch;

α_D^{20} = 184,0 for rye starch;

α_D^{20} = 183,6 for tapioca starch (see annex A);

α_D^{20} = 182,7 for wheat starch;

α_D^{20} = 181,5 for barley starch;

α_D^{20} = 181,3 for oat starch;

α_D^{20} = 184,0 for other types of starch and starch mixtures in animal feeding stuffs.

Round the result to the nearest 1 g/kg.

11 Precision

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are given in annex B. The values derived from this test may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases exceed the repeatability limit r given in or derived from Table 1.

Table 1 — Repeatability limit (r) and reproducibility limit (R)

Sample	Starch content g/kg	r g/kg	R g/kg
Maize gluten meal	190,4	12,5	20,2
Piglet feed	347,1	12,7	27,5
Layer feed	367,1	9,7	13,3
Peas	444,3	52,1	67,1
Dehydrated tapioca	629,3	15,0	36,1

11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment, will in not more than 5 % of cases exceed the reproducibility limit R given in or derived from Table 1.

12 Test report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, with reference to this International Standard;

— all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

— the test result obtained, or the two test results obtained if the repeatability has been checked.

Annex A
(informative)

Explanation of the specific optical rotation of potato starch and tapioca starch

A.1 Potato starch

For potato starch, three different values of the specific optical rotation appear to be used in practice.

In the original publication by Ewers (see reference [2] in the Bibliography) two values are presented for the specific optical rotation of potato starch:

α_D^{20} = 185,7° for treatment of the starch sample in a boiling water bath with 0,31 mol/l hydrochloric acid;

α_D^{20} = 195,4° for treatment of the starch sample in a boiling water bath with 0,10 mol/l hydrochloric acid.

The polarimetric determination of starch content according to reference [2] with 0,10 mol/l hydrochloric acid has not become standard practice. Usually, for the determination of starch content 0,31 mol/l hydrochloric acid is used with the corresponding specific optical rotation of 185,7°.

Unfortunately, in the past these two values from the original publication [2] have been confused in publications of the European Community (EC) (see Bibliography) mentioning the specific optical rotation of potato starch. This has caused confusion.

In the Official Journal of the European Communities in 1967 (reference [3]), the correct value of 185,7° was presented. After revision of the EC protocol in 1972, the wrong value of 195,4° was mentioned in the Official Journal [4].

In 1980, in the Official Journal [5], an incorrect rectification of the specific optical rotation of potato starch was published: 195,4° was changed to 185,4°. However, the latter should have been 185,7°.

In 1987, the incorrect value of 195,4° introduced by the EC was adopted by the Analytical Working Party of the Starch Experts Group (STEX) of the European Starch Association (ESA) in reference [6].

Afterwards, the value wrongly rectified by the EC as 185,4°, was adopted in ISO/CD 10520. In the subsequent ISO/DIS 10520 (1994) the correct value of 185,7° was presented. Although the wrong value of 185,4 ° was introduced again in the second ISO/DIS 10520.2 (1995), the published version of ISO 10520:1997 [7] contains the correct value of 185,7°.

A.2 Tapioca starch

The value of 183,6° for the specific optical rotation of tapioca starch has been adopted from reference [8].