INTERNATIONAL STANDARD

ISO 10520

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Native starch — Determination of starch content — Ewers polarimetric method

Amidons et fécules natifs — Dosage de l'amidon — Méthode polarimétrique de Ewers

Reference number
ISO 10520:1997(E)

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.

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.

International Standard ISO 10520 was prepared by Technical Committee ISO/TC 93, *Starch (including derivatives and by-products).*

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

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Native starch — Determination of starch content — Ewers polarimetric method

1 Scope

This International Standard specifies a polarimetric method for the determination of the starch content of native starch, with the exception of starch with high amylose content.

It is not applicable to modified or pregelatinized (water-soluble) starch.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 1666:1996, *Starch — Determination of moisture content — Oven-drying method*

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

3 Principle

The method includes two intermediate determination steps.

3.1 A portion of the sample is hydrolysed with dilute hydrochloric acid and the optical rotation measured polarimetrically after clarification and filtration.

3.2 A second portion of the sample is treated with 40 % (*V/V*) ethanol to extract soluble sugars and polysaccharides of lower molecular mass. The filtrate is then subjected to the procedure given in 3.1.

The difference between the measurements 3.1 and 3.2, multiplied by a factor, gives the starch content of the sample.

NOTE — Key parameters of the method are the time and temperature of the hydrolysis, and the correct use and calibration of the polarimeter. Consequently, the method includes constant agitation in the water bath, which should be of a size appropriate to ensure rapid temperature rise and steady temperature conditions.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and water complying with grade 2 in accordance with ISO 3696.

4.1 Dilute hydrochloric acid, c(HCl) = 7,7 mol/l

Dilute 63,7 ml of hydrochloric acid (ρ_{20} = 1,19 g/ml) with water up to 100 ml.

4.2 Dilute hydrochloric acid, c(HCl) = 0,309 mol/l

Dilute 25,6 ml of hydrochloric acid (ρ_{20} = 1,19 g/ml) with water up to 1 000 ml.

NOTE —The concentration should be verified using sodium hydroxide solution [c(NaOH) = 0,1 mol/l] and Methyl red as indicator: 10 ml HCl should consume 30,94 ml of 0,1 mol/l NaOH.

4.3 Dilute ethanol, 40 % (*V/V*) (ρ_{20} = 0,948 g/ml)

4.4 Carrez solution I

Dissolve 10,6 g of potassium hexacyanoferrate(II) trihydrate [$K_4[Fe(CN)_6].3H_2O$] in water. Dilute to 100 ml with water.

4.5 Carrez solution II

Dissolve 21,9 g of zinc acetate dihydrate [$Zn(CH_3COO)_2.2H_2O$] and 3 g of glacial acetic acid in water. Dilute to 100 ml with water.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Volumetric flasks, of capacity 100 ml.

5.2 Shaking boiling water bath, or boiling water bath equipped with a magnetic stirrer.

5.3 Polarimeter, adjusted to a wavelength of 589,3 nm, with 200 mm tubes.

5.4 Analytical balance, capable of weighing to the nearest 0,001 g.

6 Preparation of test sample

If the particle size of the laboratory sample exceeds 0,5 mm, grind the sample to pass a sieve with 0,5 mm apertures. Homogenize the sample thus prepared.

7 Procedure

Carry out weighings to the nearest 0,001 g (see 5.4).

7.1 Determination of optical rotation of a total portion

7.1.1 Weigh 2,5 g ± 0,05 g (m_1) of the test sample and transfer it to a volumetric flask (5.1). Add 25 ml of the dilute hydrochloric acid (4.2) and agitate to distribute the test sample evenly. Add a further 25 ml of the dilute hydrochloric acid (4.2).

7.1.2 Immerse the flask in the boiling water bath (5.2) and shake continuously or immerse the flask in the boiling water bath equipped with a magnetic stirrer and stir at minimum speed.

7.1.3 Leave the flask for 15 min ± 5 s in the boiling water bath and stop shaking or stirring shortly before removing it. Immediately add 30 ml of cold water and cool rapidly under flowing water to 20 °C ± 2 °C

7.1.4 Add 5 ml of the Carrez solution I (4.4) and shake for 1 min.

7.1.5 Add 5 ml of the Carrez solution II (4.5) and shake for 1 min.

7.1.6 Dilute to the mark with water. Homogenize and filter the solution through a suitable filter funnel and paper. If the filtrate is not perfectly clear, repeat the operations with 10 ml of each of the Carrez solutions.

7.1.7 Measure the optical rotation (α_1) of the solution in a 200 mm tube with the polarimeter (5.3).

7.2 Determination of optical rotation of substances soluble in 40 % (*V/V*) ethanol

7.2.1 Weigh 5 g ± 0,1 g (m_2) of the sample and transfer to a 100 ml volumetric flask (5.1). Add about 80 ml of the ethanol solution (4.3). Leave the flask to stand for 1 h at room temperature; shake vigorously six times during the hour to ensure thorough mixing of the test sample with the ethanol. Dilute to 100 ml with ethanol (4.3), homogenize and filter.

7.2.2 Pipette 50 ml of the filtrate (equivalent to 2,5 g of the test portion) into a volumetric flask (5.1). Add 2,1 ml of the dilute hydrochloric acid (4.1) and shake vigorously.

Fit a reflux condenser to the flask and immerse the flask in a boiling water bath.

Remove the flask from the boiling water bath after 15 min ± 5 s.

Cool to 20 °C ± 2 °C.

7.2.3 Clarify the solution using Carrez I and II solutions as in 7.1.4, 7.1.5 and then continue as in 7.1.6.

7.2.4 Measure the optical rotation (α_2) of the solution as in 7.1.7.

7.3 Determination of dry matter content

Determine the moisture content of the test sample, w_0, in accordance with the method given in ISO 1666. Then calculate the dry matter content, w_1, of the test sample using the following equation:

$$w_1 = 100 - w_0$$

8 Expression of results

Calculate the starch content of the dry matter content of the test sample, w, as a percentage by mass, using the following equation:

$$w = \frac{2000}{\alpha_D^{20}} \times \left[\frac{2{,}5\alpha_1}{m_1} - \frac{5\alpha_2}{m_2} \right] \times \frac{100}{w_1}$$

where

α_1 is the numerical value of the total optical rotation measured in 7.1, in degrees;

α_2 is the numerical value of the optical rotation of the ethanol-soluble substances measured in 7.2, in degrees;

m_1 is the numerical value of the mass of the test portion in 7.1.1, in grams;

m_2 is the numerical value of the mass of the test portion in 7.2.1, in grams;

w_1 is the numerical value of the dry matter content of the test sample determined in 7.3, as a percentage by mass;

α_D^{20} is the numerical value of the specific optical rotation of pure starch measured at a wavelength of 589,3 nm, in degrees. (See table 1.)

Table 1

Starch type	Numerical value of α_D^{20} (degrees)
Rice starch	+ 185,9
Potato starch	+ 185,7
Maize starch	+ 184,6
Wheat starch	+ 182,7
Barley starch	+ 181,5
Oat starch	+ 181,3
Other types of starch and starch mixtures	+ 184,0

Round the result to one decimal place.

9 Precision

The precision of the method was established by an interlaboratory test organized by ISO/TC 93/WG 1, *Determination of starch content,* in 1990 and carried out in accordance with

ISO 5725 [1]. In this test, 12 laboratories participated. Samples investigated included maize starch, potato starch and wheat starch. The statistical results are summarized in annex A.

9.1 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, should not be greater than the repeatability limit shown in table 2 for the type of starches listed.

9.2 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different test laboratories with different operators using different equipment, should not be greater than the reproducibility limit shown in table 2 for the type of starches listed.

Table 2

Starch type	Repeatability limit *r* % (*m/m*)	Reproducibility limit *R* % (*m/m*)
Maize	2,2	4,8
Potato	1,0	7,7
Wheat	2,0	3,5
Waxy maize	1,4	8,2

10 Test report

The test report shall specify

- the method used,
- the test result(s) obtained, and
- if the repeatability has been checked, the final quoted result obtained.

It shall also mention 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 report shall include all information necessary for the complete identification of the sample.

Annex A
(informative)

Ewers method — Collaborative study 1990

Collaborative study

The calculated values for the reproducibility relative standard deviation (RSD_R) or reproducibility coefficient of variation, in relation to the concentration level, were comparable with those in the table quoted by Pocklington [4]. An equation was derived empirically by Horwitz from examination of more than 3 000 collaborative (method performance) studies.

$$RSD_R = 2^{(1 - 0{,}5 \lg c)}$$

where c is the concentration expressed as a decimal fraction.

From this equation, when $c = 1$, $RSD_R = 2$ %

It is recognized that, where values for RSD_R are in the range between 1 and 4 at this concentration, the precision is acceptable for method performance between laboratories.

The relative standard deviation within laboratories (repeatability) RSD_r is frequently between half and two-thirds of RSD_R.

In table A.1, values for RSD_R range from 1,25 % to 3,24 %.

Table A.1

Parameter	MAIZE	POTATO	WHEAT	WAXY MAIZE	MAIZE 95/ FILTER AID 5	WHEAT 90/ DEXTROSE 10	POTATO 85/SALT 15
No. of labs retained after eliminating outliers	12	11	12	11	11	12	12
No. of outliers (laboratories)	-	1	-	1	1	-	-
No. of accepted results	24	22	24	22	22	24	24
Mean value [% (*m*/*m*)]	98,2	100,0	99,0	100,0	92,7	88,9	82,2
True or accepted value [% (*m*/*m*)]	98,9	99,7	99,0	99,7	93,3	89,1	85,0
Repeatability standard deviation, s_r [% (*m*/*m*)]	0,79	0,35	0,71	0,48	0,63	0,55	1,54
Repeatability coefficient of variation (%)	0,80	0,30	0,71	0,48	0,68	0,62	1,87
Repeatability limit, $r = 2{,}8 \times s_r$ [% (*m*/*m*)]	2,22	0,98	2,00	1,37	1,78	1,57	4,34
Reproducibility standard deviation, s_R [% (*m*/*m*)]	1,69	2,73	1,24	2,90	1,17	1,95	2,66
Reproducibility coefficient of variation (%)	1,71	2,73	1,25	2,90	1,27	2,19	3,24
Reproducibility limit, $R = 2{,}8 \times s_R$ [% (*m*/*m*)]	4,77	7,72	3,50	8,20	3,33	5,52	7,53

Annex B
(informative)

Bibliography

[1] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests* (now withdrawn), was used to obtain the precision data.

[2] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results - Part 1 : General principles and definitions*

[3] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results - Part 2 : A basic method for the determination or repeatability and reproducibility of a standard measurement method*

[4] International Union of Pure and Applied Chemistry, Harmonized protocols for the adoption of standardized analytical methods and for the presentation of their performance characteristics. *J. Pure Appl. Chem.,* **62**, No 1, 1990, p.152.

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