INTERNATIONAL STANDARD

ISO 6647-1

Second edition
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Rice — Determination of amylose content —

Part 1: Reference method

Riz — Détermination de la teneur en amylose —

Partie 1: Méthode de référence

Reference number
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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.

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 documents 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).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

This second edition cancels and replaces the first edition (ISO 6647-1:2007), of which it constitutes a minor revision.

ISO 6647 consists of the following parts, under the general title *Rice — Determination of amylose content*:

— *Part 1: Reference method*

— *Part 2: Routine methods*

Rice — Determination of amylose content —

Part 1: Reference method

1 Scope

This part of ISO 6647 specifies a reference method for determining calibration values for standards that will be used to make a standard curve for the quantification of amylose content in milled, non-parboiled rice in the range of amylose content from 0 % to 30 %.

2 Normative references

No normative references cited in this document.

3 Terms and definitions

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

3.1
amylose
molecules consisting of linear chains containing more than 200 linked glucose units

3.2
amylopectin
molecules consisting of branched chains ranging from 6 to 100 linked glucose units

3.3
waxy rice
waxy rice contains no chains of length consistent with being amylose

4 Principle

The linear chains of starch are separated on the basis of hydrodynamic volume and molecular weight by size exclusion chromatograph.[2] Flour is gelatinised in a solution of sodium hydroxide and the molecules of starch in the solution are debranched with isoamylase,[1][2] The linear chains are separated by size exclusion chromatography (SEC), and the proportion of amylose chains is calculated by the area under the amylose peak relative to the full detector response.

5 Reagents

All the reagents used shall be of recognized analytical quality and the water used shall be distilled, or demineralised water, or water of equivalent purity.

5.1 Ethanol, 95 % (v/v).

5.2 Sodium hydroxide, 0,25 mol/l solution.

5.3 Glacial acetic acid.

5.4 Sodium acetate buffer, 0,2 mol/l solution, brought to pH4 with glacial acetic acid. Mix 10 ml buffer with additional 360 µl glacial acetic acid.

5.5 Isoamylase.

5.6 Ion exchange mixed bed resin, for example, AG 501-X8 and Bio-Rex MSZ 501(D)[1)].

5.7 Ammonium acetate eluant, 0,05 mol/l solution, pH 4,75.

6 Apparatus

The usual laboratory apparatus and, in particular, the following:

6.1 Stirrer and stirring magnets.

6.2 Hot plate.

6.3 Microcentrifuge.

6.4 Microcentrifuge tubes, 2,0 ml capacity.

6.5 Scintillation vials, 20 ml capacity.

6.6 Vortex mixer.

6.7 Water baths, capable of holding 50 °C and of reaching boiling point.

6.8 Analytical balance, capable of weighing to the nearest 0,000 1 g.

6.9 Pipettes and micropipettes, capacity of 0,2 ml, 1 ml, 5 ml, and 10 ml.

6.10 Syringe, capable of delivering 25 µl.

6.11 Size Exclusion Chromatograph (SEC) fitted with a refractive index (RI) detector.

6.12 SEC column, suitable for separating chains of MW < 1 620 000.

6.13 Grinder, capable of reducing uncooked milled rice to flour which will pass through a 150 µm to 180 µm (80 to 100 Mesh) sieve. A cyclone mill with 0,5 mm screen is recommended.

6.14 Sieve, size 150 µm to 180 µm (80 to 100 Mesh).

7 Sampling

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

Sampling is not part of the method specified in this part of ISO 6647. A recommended sampling method is given in ISO 24333.

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1) AG 501-X8 and Bio-Rex MSZ 501 (D) are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

8 Procedure

8.1 Preparation of test samples

The calibration is carried out using 300 g of flour of each of the five standards from specific varieties[2)], each standard carrying one of the five alleles of the *Waxy* gene, which is the gene responsible for amylose synthesis.

In the grinder (6.13), grind at least 10 g of milled rice of each sample to very fine flour which will pass through the sieve (6.14).

8.2 Test portion and preparation of the solutions

Weigh triplicate samples of 50 mg ± 0,5 mg of each test sample into glass scintillation vials of known weight (6.5). To this, carefully add a small magnetic stirrer (6.1), as well as 0,5 ml of ethanol (5.1), using a pipette (6.9), washing down any of the test portion adhering to the side of the vial. Shake slightly in order to wet all of the sample. Pipette 2,0 ml of sodium hydroxide solution (5.2) and mix. Disperse starch completely by heating the mixture to a gentle boil on a hot plate (6.2) for about 10 min, ensuring mixture does not bubble over. When the solution becomes clear (about 10 min of boiling gently), remove it from the hot plate. Weigh each vial and adjust the weight to 4 g with water that has been heated to 60 °C to 70 °C.

8.3 Debranching to obtain linear chains of starch

Transfer 800 µl of each gelatinised solution to a microcentrifuge tube (6.4) by using the appropriate pipette (6.9). Add 200 µl of the sodium acetate buffer (5.4). Add 2,5 activity units (U) isoamylase using the syringe (6.10). Mix well using a vortex mixer (6.6). Incubate for 2 h in a water bath (6.7) at 50 °C, agitating every half hour, then boil for 5 min to denature the isoamylase. Transfer supernatant to a clean microcentrifuge tube and add ~0,1 g ion exchange resin (5.6). Incubate at 50 °C for 30 min, then centrifuge. Carefully transfer supernatant to a clean microcentrifuge tube. The sample is now ready to be injected into an SEC (6.11) and should be injected the day of preparation.

8.4 Blank test

Carry out a blank test in parallel with the determination, by the same procedure, using the same quantities of all the reagents as in 8.2 and 8.3, but using 800 µl of the sodium hydroxide solution (5.2) that does not contain gelatinised starch.

8.5 Operating conditions of SEC

The SEC (6.11) should comprise a separation module, a refractive index detector, software, and a suitable column (6.12). If an autosampler is used, it is preferable that it contains a sample heater to maintain the debranched starch samples at 40 °C. Ensure that the SEC system and needle wash are primed, the injector is purged, the seals are washed, and the column is equilibrated according to the operating instructions of the SEC system and column provider.

For the analysis of starch, eluant (5.7) should be flowing at 0,5 ml/min through an appropriate size exclusion column (6.12). The column (6.12) is maintained at 60 °C. Once the column is calibrated and the baseline of the RI (6.11) is stable, samples can be injected. Each sample and blank is run for 40 min.

8.6 Calculation of amylose values

Once the SEC runs are completed, determine the peak for amylose chains based on the peak missing from the sample that is waxy and which does not contain amylose. After fixing baseline issues and normalizing

2) Standard rice flours are available from the International Rice Research Institute, DAPO 7777, Metro Manila, Philippines. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO. Equivalent products may be used if they can be shown to lead to the same results.

the detector response, calculate the area under each peak associated with starch, and then the area under the peak associated with amylose chains. The percent amylose follows the following formula:

$$A = \frac{Sa}{St} \times 100\,\% \quad (1)$$

where

A is the percent amylose;

Sa is the area under the amylose peak;

St is the total area under the starch peaks.

9 Expression of results

The amylose peak is identified by comparing with the SEC trace of a waxy variety because these varieties do not contain amylose. The amylose content is calculated for each of the three replicates for each of the five samples by taking the arithmetic mean of the three replicates as the amylose value. Any replicate that is significantly different from the others should be repeated completely from 8.1.

10 Precision

10.1 Interlaboratory test

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

10.2 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 be greater than the repeatability limit *r* (*r* will be deduced from the results of the interlaboratory test).

10.3 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 be greater than the reproducibility limit *R* (*R* will be deduced from the results of the interlaboratory test).

11 Test report

The test report shall specify the following:

a) all information necessary for the complete identification of the sample;

b) the sampling method used;

c) the test method used, with reference to this part of ISO 6647 (i.e. ISO 6647-1);

d) all operating details not specified in this part of ISO 6647, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained or, if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Results of an interlaboratory test

An international interlaboratory test involving seven laboratories was carried out on five samples grown at the International Rice Research Institute (IRRI) (Los Baños, Philippines). The test was organized by the International Network for Quality Rice. Each laboratory determined amylose content by SEC and returned their raw SEC data to IRRI. The results were analysed in accordance with ISO 5725-1 and ISO 5725-2 to give the precision data shown in Table A.1 and Figure A.1.

Table A.1 — Precision data

Parameter	Samples				
	A	B	C	D	E
Number of laboratories after elimination outliers	6	7	7	7	7
Mean amylose content, % m/m	0,00	4,29	11,81	18,23	24,53
Repeatability standard deviation (S_r), %	0,00	0,18	0,24	0,16	0,13
Repeatability limit r ($r = 2{,}77 \times S_r$), %	0,00	0,53	0,91	1,77	0,39
Reproducibility standard deviation (S_R), %	0,00	0,70	0,86	0,61	1,23
Reproducibility limit R ($R = 2{,}77 \times S_R$), %	0,00	1,94	2,38	1,69	2,22

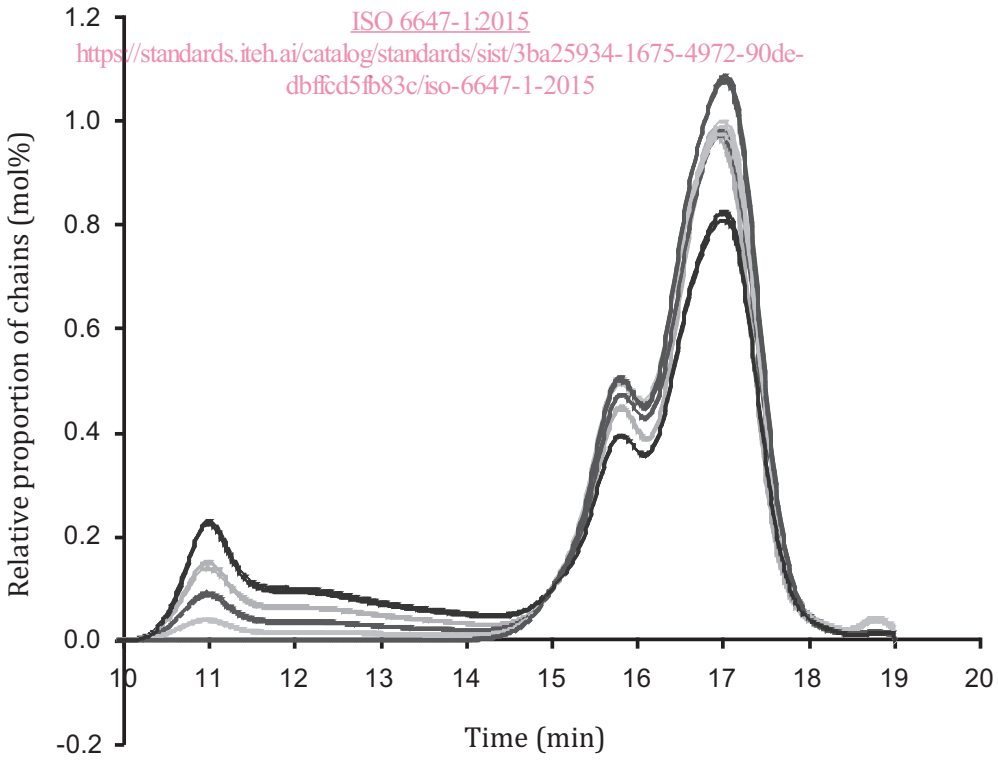

Figure A.1 — Example of SEC traces