INTERNATIONAL STANDARD

ISO 6647-2

Second edition
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Rice — Determination of amylose content —

Part 2: Routine methods

Riz — Détermination de la teneur en amylose —

Partie 2: Méthodes de routine

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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

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 6647-2:2007), 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 2:
Routine methods

1 Scope

This part of ISO 6647 specifies a simplified routine method for the determination of the amylose content of milled, non-parboiled rice in the range from 1 % to 30 %. Rice samples for which the amylose content has been determined by the reference method size exclusion chromatography (SEC) are used as standards to generate the calibration curve.

NOTE The use of standards calibrated by SEC is an approach to determine the true amylose content and decreases the conversion errors of this part of ISO 6647.[1]

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6647-1, *Rice — Determination of amylose content — Part 1: Reference method*

ISO 7301, *Rice — Specification*

ISO 8466-1, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 6647-1 and ISO 7301 apply.

4 Principle

Rice is ground to a very fine flour to break up the endosperm structure in order to aid complete dispersion and gelatinisation. A test portion is dispersed in sodium hydroxide solution, then an aliquot is mixed with iodine solution. The absorbance, at 620 nm or 720 nm of the colour complex formed, is then determined using a spectrophotometer.

The amylose content of the sample is then read from a calibration graph, which is prepared using rice samples with known amylose content, determined using the reference method (see ISO 6647-1).

NOTE Rice samples with certified amylose content according to ISO 6647-1 are used as standards.

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, 1 mol/l solution.

5.3 Sodium hydroxide, 0,09 mol/l solution.

5.4 Acetic acid, 1 mol/l solution.

5.5 Iodine solution.

Weigh, to the nearest 5 mg, 2,000 g of potassium iodide in a weighing bottle fitted with a stopper. Add sufficient water to form a saturated solution. Add 0,200 g of iodine, weighed to the nearest 1 mg. When all the iodine has dissolved, transfer the solution quantitatively to a 100 ml volumetric flask (6.4), make up to volume with water, and mix.

Prepare a fresh solution on each day of use and protect it from light.

6 Apparatus

Usual laboratory equipment and, in particular, the following:

6.1 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.

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

6.3 Spectrophotometer, with matching cells, usually of 1 cm path length, capable of measuring absorbance at 600 nm - 720 nm, and cuvettes.

6.4 Volumetric flasks, 100 ml.

6.5 Boiling water bath.

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

6.7 Test tubes, 20 ml.

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

6.9 Conical flask, 100 ml.

6.10 Vortex mixer.

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.

8 Procedure

8.1 Preparation of test samples

In the grinder (6.1), grind at least 10 g of milled rice of each sample to very fine flour which will pass through the sieve (6.2).

8.2 Test portion and preparation of the test solution

Weigh 100 mg ± 0.5 mg of the test sample into a 100 ml conical flask (6.9). To this test portion, carefully add 1,0 ml of ethanol (5.1) using a pipette, washing down any of the test portion adhering to the side of the flask. Shake slightly in order to wet all of the sample. Add 9,0 ml of sodium hydroxide solution (5.2) from a pipette and mix. Disperse starch completely by either heating the mixture in a boiling water bath (6.5) for 10 min or by standing overnight in covered flasks. Allow cooling to room temperature (if boiled) and transfer to 100 ml volumetric flask (6.4). Vortex (6.10), make up to volume with water, and then vortex again (6.10).

8.3 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 the determination, but using 0,50 ml of sodium hydroxide solution (5.3) instead of the test solution.

8.4 Preparation of the calibration graph

8.4.1 Preparation of the set of calibration solutions

Use five calibrated rice samples[1], with a distribution of amylose content from 0 % to 30 %, and for which the amylose content has been certified by the reference method in ISO 6647-1. Alternatively, create a set of standards from different rice varieties calibrated against a standard curve made from the calibrated standards.

Prepare the calibration solutions as in 8.1 and 8.2.

8.4.2 Colour development and spectrometric measurements

Pipette a 0,50 ml aliquot of each calibration solution into a series of five test tubes (6.7). Add 5,00 ml of water, 0,10 ml of acetic acid (5.4), and 0,20 ml of iodine solution (5.5). Place an additional 4,20 ml of water into the tube to make the volume of the reaction mixture up to 10,00 ml. Cover the tube and mix well on a vortex mixer (6.10) or by inverting several times.

Measure the absorbance at either 620 nm or 720 nm (choose the wavelength used in ISO 6647-1) against the blank (8.3), immediately after mixing, using the spectrophotometer (6.3).

8.4.3 Plotting the calibration graph

Prepare a calibration graph by plotting absorbance against the amylose content, expressed as a percentage by mass, in the milled rice on the dry basis.

8.5 Determination

Pipette a 0,50 ml aliquot of the test solution (8.2) into a test tube (6.7). Add 5,00 ml of water, 0,10 ml of acetic acid (5.4), and 0,20 ml of iodine solution (5.5). Put an additional 4,20 ml of water into the tube to make the volume of the reaction mixture up to 10,00 ml. Cover the tube and mix well on a vortex mixer

1) Calibrated rice samples can be obtained from the International Rice Research Institute. 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.

(6.10) for 2 min or by inverting at least three times. Measure the absorbance at either 620 nm or 720 nm against the blank (8.3), immediately after mixing, using the spectrometer (6.3). Ensure that the standards and test solutions are measured at the same wavelength, either 620 nm or 720 nm, and in the same run.

Carry out two determinations on separate test portions taken from the same test sample.

NOTE If double determinations are made, based on two independent preparations of the sample (8.1), this should be noted in the test report.

9 Expression of results

The amylose content, expressed as a percentage by mass on dry basis, is obtained by referring the absorbance (8.5) to the calibration graph (8.4.3) according to ISO 8466-1.

Take the result of the arithmetic mean of the two determinations.

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. Annex B shows a comparison between amylose values determined with the standard curve in the previous version of this standard, and the standard curve used here, which is calibrated to SEC values.

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:

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 6647-2);

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 12 laboratories in 12 countries was carried out on 15 triplicate samples of rice spanning the range of amylose content found in rice. The test was organized by the International Network for Quality Rice and the International Rice Research Institute (IRRI) and the results obtained were subjected to statistical analysis in accordance with ISO 5725-1 and ISO 5725-2 to give the precision data shown in Table A.1 and Figure A.1. Samples and standards were measured at 620 nm. Samples and calibrated standards, as per ISO 6647-1, were provided by IRRI.