



SLOVENSKI STANDARD
oSIST prEN ISO 6647-2:2019

01-december-2019

Riž - Določevanje amiloze - 2. del: Rutinska metoda: spektrofotometrijska rutinska metoda brez postopka razmaščevanja in s kalibracijo v skladu s standardi za riž (ISO/DIS 6647-2:2019)

Rice - Determination of amylose content - Part 2: Routine method: Spectrophotometric routine method without defatting procedure and with calibration from rice standards (ISO/DIS 6647-2:2019)

Reis - Bestimmung des Amylosegehalts - Teil 2: Routinemethode: Spektrophotometrische Routinemethode ohne Entfettungsverfahren und mit Kalibrierung nach Reisstandards (ISO/DIS 6647-2:2019)

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Riz - Détermination de la teneur en amylose - Partie 2: Méthode de routine : méthode spectrophotométrique de routine sans mode opératoire de dégraissage et avec étalonnage à l'aide d'étalons de riz (ISO/DIS 6647-2:2019)

Ta slovenski standard je istoveten z: prEN ISO 6647-2

ICS:

67.060	Žita, stročnice in proizvodi iz njih	Cereals, pulses and derived products
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oSIST prEN ISO 6647-2:2019

en

DRAFT INTERNATIONAL STANDARD

ISO/DIS 6647-2

ISO/TC 34/SC 4

Secretariat: SAC

Voting begins on:
2019-10-21Voting terminates on:
2020-01-13

Rice — Determination of amylose content —

Part 2:

Routine method: Spectrophotometric routine method without defatting procedure and with calibration from rice standards

ICS: 67.060

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ISO/CEN PARALLEL PROCESSING



Reference number
ISO/DIS 6647-2:2019(E)

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Published in Switzerland

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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 of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

This third edition cancels and replaces the second edition (ISO/DIS 6647-2:2015), which has been technically revised.

A list of all parts in the ISO 6647 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Rice — Determination of amylose content —

Part 2:

Routine method: Spectrophotometric routine method without defatting procedure and with calibration from rice standards

1 Scope

This Annex specifies two simplified routine methods for the determination of the amylose mass fraction of milled rice, non-parboiled. The main difference between the two methods is the dispersion procedure: method A specifies hot, and method B cold, dispersion.

Both methods are applicable to rice with an amylose mass fraction higher than 5 %.

Note These methods describe simplified procedures for the preparation of samples, which are frequently used in routine laboratories. The methods use the same reagents as the reference method (see ISO 6647-1), but omit the defatting step. Rice samples of which the amylose mass fraction has been determined by the reference method are used as standards.

2 Normative references

The following referenced documents are indispensable for the application 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 712, *Cereals and cereal products — Determination of moisture content — Reference method*

ISO 6647-1, *Rice — Determination of Amylose content — Reference method - Part 1: Spectrophotometric method with a defatting procedure by methanol and with calibration solutions of potato amylose and waxy rice amylopectin*

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 gelatinization. A test portion is dispersed in a sodium hydroxide solution, to an aliquot portion of which is added an iodine solution. The absorbance, at 720 nm, of the colour complex formed is then determined using a spectrophotometer.

Measurement wavelengths of 620 nm or 680 nm can also be used.

The amylose mass fraction of the sample is then read from a calibration graph, which is prepared by using rice samples of known amylose mass fraction, determined using the reference method specified above.

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5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

5.1 Ethanol, a volume fraction of 95 %.

5.2 Sodium hydroxide

1 mol/l solution, for method A.

2 mol/l solution, for method B.

5.3 Sodium hydroxide

0,09 mol/l solution, for method A.

0,18 mol/l solution, for method B.

5.4 Acetic acid, 1 mol/l solution.

5.5 Iodine solution

Weigh (6.8), 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

The usual laboratory apparatus 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 (100 mesh to 80 mesh) sieve. A cyclone mill with 0,5 mm screen is recommended.

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

6.3 Spectrophotometer, with matching cells, usually of pathlength 1 cm, capable of measuring absorbance at 720 nm (or 620 nm or 680 nm).

6.4 Volumetric flasks, 100 ml.

6.5 Boiling water bath, for method A only

6.6 Magnetic stirrer, capable of stirring at 950 r/min to 1 000 r/min, for method B only.

6.7 Conical flasks, 100 ml.

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

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

7 Sampling

A representative sample should have been sent to the laboratory. It should not have 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^[3].

8 Procedure

8.1 Determination of moisture

On a separate portion of the laboratory sample and the standard samples, carry out a moisture determination in accordance with ISO 712.

8.2 Preparation of test sample

In the grinder (6.1) grind at least 4 g of milled rice which will pass through the sieve (6.2).

8.3 Test portion and preparation of the test solution

8.3.1 Weigh (6.8) 100 mg \pm 0,5 mg of the test sample (8.2) into a 100 ml conical flask (6.7). To this test portion carefully add 1 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 the entire sample.

8.3.2 Method A

Pipette (6.9) 9,0 ml of sodium hydroxide solution (5.2.1) into the conical flask and mix. Then heat the mixture on a boiling water bath (6.5) for 10 min to disperse the starch. Allow to cool to room temperature and transfer quantitatively to a 100 ml volumetric flask (6.4). Make up to volume with water and mix vigorously.

8.3.3 Method B

Pipette (6.9) 9,0 ml of sodium hydroxide solution (5.2.2) into the conical flask and mix. Stir the mixture using a magnetic stirrer (6.6) for 10 min to obtain the dispersion. Remove the stirrer and transfer quantitatively to a 100 ml volumetric flask (6.4). Make up to volume with water and mix vigorously.

It is recommended to swirl the liquid in the volumetric flask before adding the water and after making up to volume.

8.4 Preparation of the blank solution

Prepare a blank solution using the same procedure and the same quantities of all the reagents as in the determination, but using 5,0 ml of sodium hydroxide solution (5.3.1 for method A and 5.3.2 for method B) instead of the test solution.

8.5 Preparation of the calibration graph

8.5.1 Preparation of the set of calibration solutions

Select at least four rice samples with a distribution of amylose mass fraction in the measured range. For each sample, ensure that the amylose mass fraction has been determined by the reference method specified in ISO 6647-1 independently, 20 times.

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Alternatively, a certified reference material may be used. Prepare the calibration solutions as in [8.2](#) and [8.3](#).

8.5.2 Colour development and spectrophotometric measurements

Pipette ([6.9](#)) a 5,0 ml aliquot of each calibration solution into a series of five volumetric flasks ([6.4](#)) each containing about 50 ml of water. Pipette ([6.9](#)) 1,0 ml of acetic acid ([5.4](#)) for method A or 2,0 ml for method B and mix. Then pipette ([6.9](#)) 2,0 ml of iodine solution ([5.5](#)), make up to the mark with water and mix. Allow to stand for 10 min.

Measure the absorbance at 720 nm against the blank solution ([8.4](#)) using the spectrophotometer ([6.3](#)). Measurement wavelengths of 620 nm or 680 nm can also be used (see [Annex A](#)).

8.5.3 Plotting the calibration graph.

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

Instead of manual spectrometric measurements, an automatic analyzer, e.g. a flow injection analyzer, may be used (see ISO 6647-1:2019, Annex C).

8.6 Determination

Pipette ([6.9](#)) a 5,0 ml aliquot of the test solution ([8.3](#)) into a volumetric flask ([6.4](#)) containing about 50 ml of water and proceed according to [8.5.2](#), starting with the addition of acetic acid ([5.4](#)).

Measure the absorbance at 720 nm (or at 620 nm or at 680 nm, see [Annex A](#)) against the blank solution ([8.4](#)) using the spectrophotometer ([6.3](#)).

Instead of manual spectrometric measurements, an automatic analyser, e.g. a flow injection analyzer, may be used (see ISO 6647-1:2019, Annex C).

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

If double determinations are made, based on two independent preparations of the sample ([8.2](#)), this should be noted in the test report.

9 Expression of results

The amylose mass fraction, expressed as a percentage on the dry basis, is obtained by referring the absorbance ([8.6](#)) to the calibration graph ([8.5.3](#)) in accordance with ISO 8466-1.

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

Any result given should clearly refer to the method used (i.e. whether, for calibration, amylose solutions or rice samples analyzed in accordance with ISO 6647-1 have been used).

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.