INTERNATIONAL STANDARD

**ISO 6647-2**

Third edition
2020-07

# Rice — Determination of amylose content —

## Part 2: Spectrophotometric routine method without defatting procedure and with calibration from rice standards

*Riz — Détermination de la teneur en amylose —*

*Partie 2: Méthode spectrophotométrique de routine sans mode opératoire de dégraissage et avec étalonnage à l'aide d'étalons de riz*



Reference number
ISO 6647-2:2020(E)

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Published in Switzerland

 

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# Foreword

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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).

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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*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 338, *Cereal and cereal products,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This third edition cancels and replaces the second edition (ISO 6647-2:2015), which has been technically revised. The main changes compared with the previous edition are as follows.

— The set of calibration solutions in ISO 6647-2:2015 were made by rice samples analysed by size exclusion chromatography. In this document, the set of calibration solutions are made by rice samples analysed by spectrophotometer UV-VIS, without delipidization.

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

## 1 Scope

This document 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 dispersion, and method B specifies 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 where the amylose mass fraction has been determined by the reference method are used as standards.

## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 6647-1:2020, *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.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

## 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. An aliquot portion is taken to 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.

## 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:**

a) 1 mol/l solution, for method A.

b) 2 mol/l solution, for method B.

**5.3 Sodium hydroxide for blank solution:**

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

b) 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

Usual laboratory apparatus and, in particular, the following.

**6.1 Grinder**, capable of reducing uncooked milled rice to flour that 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 path length 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 document. A recommended sampling method is given in ISO 24333.

## 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 that 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 ± 0,5 mg of the test sample (see 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 a)] 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 b)] 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 a) for method A and 5.3 b) 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.

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 (see 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 analyser, e.g. a flow injection analyser, may be used (see ISO 6647-1:2020, Annex C).

## 8.6 Determination

Pipette (6.9) a 5,0 ml aliquot of the test solution (see 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 (see 8.4) using the spectrophotometer (6.3).

Instead of manual spectrometric measurements, an automatic analyser, e.g. a flow injection analyser, may be used (see ISO 6647-1:2020, 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 (see 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, shall be obtained by referring the absorbance (see 8.6) to the calibration graph (see 8.5.3) in accordance with ISO 8466-1.

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

Any result given should clearly refer to the method used (i.e. whether, for calibration, amylose solutions or rice samples analysed 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.

## 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 that 5 % of cases exceed the repeatability limit, $r_{720}$, expressed as a percentage by mass, calculated from Formulae (1) and (2):

Method A:

$$r_{720} = 22,47 \times \frac{1}{\bar{w}^{0,66}} \tag{1}$$

Method B:

$$r_{720} = 24,01 \times \frac{1}{\bar{w}^{0,61}} \tag{2}$$

where

$\bar{w}$ is the mean of the two mass fraction results, expressed in grams per 100 g;

720 is the wavelength, in nanometres, at which the absorbance was measured.

## 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, $R_{720}$, expressed as a percentage by mass, calculated from Formulae (3) and (4):

Method A:

$$R_{720} = 50,55 \times \frac{1}{\bar{w}^{0,68}} \tag{3}$$

Method B:

$$R_{720} = 83,11 \times \frac{1}{\bar{w}^{0,63}} \tag{4}$$

where

$\bar{w}$ is the mean of the two mass fraction results, expressed in grams per 100 g;

720 is the wavelength, in nanometres, at which the absorbance was measured.

## 11 Test report

The test report shall at least specify the following:

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

b) the sampling method used, if known;

c) the test method used (A or B), with reference to this document, i.e. ISO 6647-2:2020;

d) all operating details not specified in this document, or regarded as optional, together with details of any incidents that could have influenced the test result(s);

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

f) the date of the test.

# Annex A
(informative)

# Results of an interlaboratory test for the routine method

## A.1 General

An interlaboratory test organized by FOSS Analytical AB (Sweden) in 2004, involving 23 laboratories in 11 countries including 2 international organizations, was carried out on 6 rice samples containing amylose at various mass fractions, which were provided by the Thai Industrial Standards Institute.

The results obtained were subjected to statistical analysis, which was performed by the Hungarian Standards Institution, in accordance with ISO 5725-1 and ISO 5725-2 to give the precision data shown in Tables A.1 to A.6.

## A.2 Interlaboratory test results at 720 nm

**Table A.1 — Results of the statistical evaluation for method A (hot dispersion)**

| | Rice samples | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | F |
| Number of laboratories after eliminating outliers | 21 | 21 | 22 | 22 | 22 | 19 |
| Mean mass fraction, g/100 g | 10,79 | 23,73 | 12,84 | 25,74 | 2,28 | 27,77 |
| Repeatability standard deviation, $s_r$, g/100 g | 0,52 | 0,67 | 0,51 | 0,81 | 0,30 | 0,60 |
| Coefficient of variation of repeatability, % | 4,82 | 2,84 | 3,98 | 3,14 | 13,06 | 2,16 |
| Repeatability limit, $r = 2{,}8s_r$, g/100 g | 1,46 | 1,89 | 1,43 | 2,27 | 0,84 | 1,68 |
| Reproducibility standard deviation, $s_R$, g/100 g | 1,07 | 1,54 | 1,15 | 1,48 | 0,66 | 1,33 |
| Coefficient of variation of reproducibility, % | 9,95 | 6,47 | 8,93 | 5,75 | 28,86 | 4,80 |
| Reproducibility limit, $R = 2{,}8s_R$, g/100 g | 3,01 | 4,30 | 3,21 | 4,14 | 1,85 | 3,73 |

**Table A.2 — Results of the statistical evaluation for method B (cold dispersion)**

| | Rice samples | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | F |
| Number of laboratories after eliminating outliers | 20 | 20 | 18 | 20 | 21 | 20 |
| Mean mass fraction, g/100 g | 10,99 | 23,48 | 13,35 | 26,15 | 1,98 | 27,89 |
| Repeatability standard deviation, $s_r$, g/100 g | 0,50 | 0,97 | 0,53 | 0,87 | 0,35 | 0,99 |
| Coefficient of variation of repeatability, % | 4,58 | 4,15 | 3,95 | 3,33 | 17,83 | 3,54 |
| Repeatability limit, $r = 2{,}8s_r$, g/100 g | 1,41 | 2,73 | 1,48 | 2,44 | 0,99 | 2,76 |
| Reproducibility standard deviation, $s_R$, g/100 g | 1,16 | 1,54 | 0,85 | 1,96 | 1,06 | 1,44 |
| Coefficient of variation of reproducibility, % | 10,55 | 6,57 | 6,40 | 7,50 | 53,70 | 5,18 |
| Reproducibility limit, $R = 2{,}8s_R$, g/100 g | 3,25 | 4,32 | 2,39 | 5,49 | 2,98 | 4,04 |

## A.3 Interlaboratory test results at 680 nm

### A.3.1 Results

**Table A.3 — Results of the statistical evaluation for method A (hot dispersion)**

| | Rice samples | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | F |
| Number of laboratories after eliminating outliers | 21 | 20 | 21 | 20 | 22 | 18 |
| Mean mass fraction, g/100 g | 11,31 | 23,71 | 13,28 | 25,83 | 2,38 | 27,69 |
| Repeatability standard deviation, $s_r$, g/100 g | 0,60 | 0,68 | 0,49 | 0,75 | 0,29 | 0,59 |
| Coefficient of variation of repeatability, % | 5,31 | 2,86 | 3,70 | 2,89 | 12,19 | 2,13 |
| Repeatability limit, $r = 2{,}8s_r$, g/100 g | 1,68 | 1,90 | 1,38 | 2,09 | 0,81 | 1,65 |
| Reproducibility standard deviation, $s_R$, g/100 g | 1,12 | 1,32 | 1,17 | 1,35 | 0,81 | 1,25 |
| Coefficient of variation of reproducibility, % | 9,94 | 5,58 | 8,78 | 5,22 | 34,11 | 4,53 |
| Reproducibility limit, $R = 2{,}8s_R$, g/100 g | 3,15 | 3,70 | 3,26 | 3,77 | 2,27 | 3,51 |

**Table A.4 — Results of the statistical evaluation for method B (cold dispersion)**

| | Rice samples | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | F |
| Number of laboratories after eliminating outliers | 18 | 16 | 18 | 18 | 19 | 19 |
| Mean mass fraction, g/100 g | 11,48 | 23,66 | 13,65 | 26,39 | 1,97 | 27,87 |
| Repeatability standard deviation, $s_r$, g/100 g | 0,47 | 0,52 | 0,56 | 0,68 | 0,37 | 0,80 |
| Coefficient of variation of repeatability, % | 4,08 | 2,22 | 4,08 | 2,58 | 18,95 | 2,86 |
| Repeatability limit, $r = 2{,}8s_r$, g/100 g | 1,31 | 1,47 | 1,56 | 1,91 | 1,05 | 2,23 |
| Reproducibility standard deviation, $s_R$, g/100 g | 0,92 | 1,12 | 0,86 | 1,44 | 1,00 | 1,42 |
| Coefficient of variation of reproducibility, % | 8,01 | 4,72 | 6,32 | 5,44 | 50,69 | 5,10 |
| Reproducibility limit, $R = 2{,}8s_R$, g/100 g | 2,57 | 3,13 | 2,42 | 4,02 | 2,80 | 3,98 |

### A.3.2 Repeatability of the methods

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 that 5 % of cases exceed the repeatability limit, $r_{680}$, expressed as a percentage by mass, calculated from Formulae (A.1) and (A.2):

Method A:

$$r_{680} = 22{,}39 \times \frac{1}{\bar{w}^{0{,}66}} \qquad \text{(A.1)}$$

Method B:

$$r_{680} = 30{,}33 \times \frac{1}{\bar{w}^{0{,}77}} \qquad \text{(A.2)}$$

where

$\bar{w}$ is the mean of the two mass fraction results, expressed in grams per 100 g;

680 is the wavelength, in nanometres, at which the absorbance was measured.