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**Rice — Determination of amylose  
content —**

Part 1:

**Spectrophotometric method with a  
defatting procedure by methanol and  
with calibration solutions of potato  
amylose and waxy rice amylopectin**

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*Riz — Détermination de la teneur en amylose —*

*Partie 1: Méthode spectrophotométrique avec un mode opératoire de  
dégraissage au méthanol et des solutions d'étalonnage d'amylose de  
pomme de terre et d'amylopectine de riz gluant*



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CP 401 • Ch. de Blandonnet 8  
CH-1214 Vernier, Geneva  
Phone: +41 22 749 01 11  
Email: [copyright@iso.org](mailto:copyright@iso.org)  
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# Contents

	Page
Foreword.....	iv
<b>1 Scope</b> .....	<b>1</b>
<b>2 Normative references</b> .....	<b>1</b>
<b>3 Terms and definitions</b> .....	<b>1</b>
<b>4 Principle</b> .....	<b>2</b>
<b>5 Reagents</b> .....	<b>2</b>
<b>6 Apparatus</b> .....	<b>3</b>
<b>7 Sampling</b> .....	<b>4</b>
<b>8 Procedure</b> .....	<b>4</b>
8.1 Preparation of test sample.....	4
8.2 Test portion and preparation of the test solution.....	4
8.3 Preparation of the blank solution.....	4
8.4 Preparation of the calibration graph.....	4
8.4.1 Preparation of the set of calibration solutions.....	4
8.4.2 Colour development and spectrophotometric measurements.....	5
8.4.3 Plotting the calibration graph.....	5
8.5 Determination.....	5
<b>9 Expression of results</b> .....	<b>5</b>
<b>10 Precision</b> .....	<b>5</b>
10.1 Interlaboratory test.....	5
10.2 Repeatability.....	6
10.3 Reproducibility.....	6
<b>11 Test report</b> .....	<b>6</b>
<b>Annex A (informative) Determination of the quality of the potato amylose stock suspension</b> .....	<b>7</b>
<b>Annex B (informative) Results of an interlaboratory test</b> .....	<b>9</b>
<b>Annex C (informative) Example of a flow injection analyser (FIA) for the determination of amylose</b> .....	<b>10</b>
<b>Bibliography</b> .....	<b>11</b>

## 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](http://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](http://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 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](http://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 6647-1:2015), which has been technically revised. The main changes compared with the previous edition are as follows.

- A spectrophotometric method with a defatting procedure by methanol and with calibration solutions of potato amylose and waxy rice amylopectin has replaced the size exclusion chromatography method.

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](http://www.iso.org/members.html).

# Rice — Determination of amylose content —

## Part 1:

# Spectrophotometric method with a defatting procedure by methanol and with calibration solutions of potato amylose and waxy rice amylopectin

## 1 Scope

This document specifies a reference method for the determination of the amylose content of milled rice, non-parboiled. The method is applicable to rice with an amylose mass fraction higher than 5 %.

This document can also be used for husked rice, maize, millet and other cereals if the extension of this scope has been validated by the user.

NOTE Amylose values determined with this document can be compared with PDO and PGI legislation.

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

ISO 15914, *Animal feeding stuffs — Enzymatic determination of total starch content*

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions 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/>

### 3.1

#### **amylose**

DEPRECATED: apparent amylose

polysaccharide constituent of starch, the macromolecules of which have glucose units linked in a predominantly linear structure

### 3.2

#### **amylopectin**

polysaccharide constituent of starch, the macromolecules of which have from 6 to 100 linked glucose units in a branched structure

## 4 Principle

Rice is ground to a very fine flour to break up the endosperm structure in order to aid complete dispersion and gelatinization; the flour is then defatted. A test portion is dispersed in a sodium hydroxide solution. An aliquot portion is taken to which an iodine solution is added. The absorbance, at 720 nm, of the colour complex formed is then determined using a spectrophotometer.

The amylose mass fraction of the sample is then read from a calibration graph, which is prepared using mixtures of potato amylose and amylopectin to make allowance for the effect of amylopectin on the colour of the amylose-iodine complex of the test solution.

## 5 Reagents

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

**5.1 Methanol**, a volume fraction of 85 %.

**5.2 Ethanol**, a volume fraction of 95 %.

**5.3 Sodium hydroxide**, 1 mol/l solution.

**5.4 Sodium hydroxide**, 0,09 mol/l solution.

**5.5 Detergent solution.**

Dissolve sodium dodecylbenzene sulfonate corresponding to a concentration of 20 g/l. Just before use, add sodium sulfite to a final concentration of 2 g/l.

**5.6 Sodium hydroxide**, for protein removal, 3 g/l solution.

**5.7 Acetic acid**, 1 mol/l solution.

**5.8 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 (6.6), make up to volume with water and mix.

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

**5.9 Stock potato amylose suspension**, free of amylopectin, 1 g/l.

**5.9.1** Defat the potato amylose by refluxing with methanol (5.1) for 4 h to 6 h in an extractor at a rate of 5 to 6 droplets per second.

The potato amylose should be pure and should be tested by amperometric or potentiometric titration. Some commercial preparations are impure and would give erroneously high results for the amylose mass fraction of rice samples. Pure amylose should bind 19 % to 20 % of its own mass of iodine. For checking the purity of amylose, see [Annex A](#).

**5.9.2** Spread the defatted potato amylose on a tray and leave for two days to allow evaporation of residual methanol and for moisture content equilibrium to be reached.

Treat the amylopectin (5.10) and the test samples (see 8.1) in the same way.

**5.9.3** Weigh (6.9) 100 mg  $\pm$  0,5 mg of the defatted and conditioned potato amylose into a 100 ml conical flask (6.8). Carefully add 1 ml of ethanol (5.2), rinsing down any potato amylose adhering to the walls of the flask. Add 9,0 ml of 1 mol/l sodium hydroxide solution (5.3) and mix. Then heat the mixture on a boiling water bath (6.7) for 10 min to disperse the potato amylose. Allow to cool to room temperature and transfer into a 100 ml volumetric flask (6.6).

Make up to volume with water and mix vigorously.

1 ml of this stock suspension contains 1 mg of potato amylose.

If the test samples, the amylose and the amylopectin are conditioned in the same environment, no correction for moisture content is necessary and the results are obtained on a dry milled rice basis. If the test samples and the stocks are not prepared under the same conditions, the moisture content of both the samples and the stocks shall be determined as specified in ISO 712 and the results should be corrected accordingly.

#### **5.10 Stock amylopectin suspension, 1 g/l.**

Prepare the stock from milled glutinous (waxy) rice with a starch content known to consist of at least 99 % by mass of amylopectin. Steep the milled glutinous rice and blend in a suitable laboratory blender (6.1) to a finely divided state. Remove protein by exhaustive extraction with a detergent solution (5.5) or, alternatively, with a sodium hydroxide solution (5.6). Wash and then defat by refluxing with methanol (5.1) as described in 5.9.1. Spread the deproteinated and defatted amylopectin on a tray and leave for two days to allow evaporation of residual methanol and for moisture content equilibrium to be reached.

Carry out the procedure given in 5.9.3, but with amylopectin instead of amylose. 1 ml of this stock suspension contains 1 mg of amylopectin.

The iodine binding capacity of amylopectin should be less than 0,2 % (see Annex A).

ISO 6647-1:2020

## **6 Apparatus** <https://standards.iteh.ai/catalog/standards/sist/b87d4c6f-e3d5-49c3-b65c-flaccb2814e7/iso-6647-1-2020>

Usual laboratory apparatus and, in particular, the following.

### **6.1 Laboratory blender.**

**6.2 Grinder**, capable of reducing uncooked milled rice to flour that will pass through a 150  $\mu$ m to 180  $\mu$ m (100 mesh to 80 mesh) sieve. A cyclone mill with 0,5 mm screen is recommended.

**6.3 Sieve**, size 150  $\mu$ m to 180  $\mu$ m (100 mesh to 80 mesh).

**6.4 Spectrophotometer**, with matching cells, usually of path length 1 cm, capable of measuring absorbance at 720 nm.

**6.5 Extraction apparatus**, capable of refluxing samples with methanol at a rate of 5 to 6 droplets per second.

**6.6 Volumetric flasks**, 100 ml.

**6.7 Boiling water bath.**

**6.8 Conical flasks**, 100 ml.

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

## 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 Preparation of test sample

In the cyclone mill (6.2), grind at least 10 g of milled rice to very fine flour that will pass through the sieve (6.3).

Defat the flour by refluxing with methanol (5.1). Follow the procedure described in 5.9.1.

NOTE Lipids compete with iodine in forming a complex with amylose and it has been shown that defatting the rice flour effectively reduces lipid interference.

After defatting, spread the flour in a thin layer in a dish or watch glass and leave for two days to allow evaporation of residual methanol and for moisture content equilibrium to be reached (see 5.9).

**WARNING — Observe safety measures, e.g. use of a fume hood, when evaporating the methanol.**

### 8.2 Test portion and preparation of the test solution

Weigh (6.9) 100 mg  $\pm$  0,5 mg of the test sample (see 8.1) into a 100 ml conical flask (6.8). To this test portion, carefully add 1 ml of ethanol (5.2), rinsing down any of the test portion adhering to the walls of the flask and shaking slightly to make all the sample wet. Add 9.0 ml of 1 mol/l sodium hydroxide solution (5.3) and mix. Then heat the mixture on a boiling water bath (6.7) for 10 min to disperse the starch. Allow to cool to room temperature and transfer to a 100 ml volumetric flask (6.6).

Make up to volume with water and mix vigorously.

### 8.3 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 0,09 mol/l sodium hydroxide solution (5.4) instead of the test solution.

### 8.4 Preparation of the calibration graph

#### 8.4.1 Preparation of the set of calibration solutions

Mix volumes of the potato amylose (5.9) and amylopectin (5.10) stock suspensions and of the 0,09 mol/l sodium hydroxide solution (5.4) in accordance with Table 1.



Table 1 — Set of calibration solutions

Amylose mass fraction in milled rice %, dry matter basis <sup>a</sup>	Potato amylose (5.9) ml	Amylopectin (5.10) ml	0,09 mol/l sodium hydroxide (5.4) ml
0	0	18	2
10	2	16	2
20	4	14	2
25	5	13	2
30	6	12	2
35	7	11	2

<sup>a</sup> These values have been calculated on the basis of an average starch mass fraction of 90 % in milled rice.

#### 8.4.2 Colour development and spectrophotometric measurements

Pipette a 5,0 ml aliquot of each calibration solution (see 8.4.1) into a series of 100 ml volumetric flasks (6.6), each containing about 50 ml of water. Add 1,0 ml of acetic acid (5.7) and mix. Then add 2,0 ml of iodine solution (5.8), 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 8.3) using the spectrophotometer (6.4).

#### 8.4.3 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.

#### 8.5 Determination

Pipette 5,0 ml aliquot of the test solution (see 8.2) into a 100 ml volumetric flask (6.6) containing about 50 ml of water and proceed in accordance with 8.4.2, starting with the addition of acetic acid (5.7).

Measure the absorbance at 720 nm against the blank solution (see 8.3), using the spectrophotometer (6.4).

Instead of manual spectrophotometric measurement, an automatic analyser, e.g. a flow injection analyser, may be used (see example given in Annex C).

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

### 9 Expression of results

The amylose mass fraction, expressed as a percentage on the dry basis, shall be obtained by referring the absorbance (see 8.5) to the calibration graph (see 8.4.3) in accordance with ISO 8466-1.

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

### 10 Precision

#### 10.1 Interlaboratory test

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