
**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*

ISO 6647-2:2020

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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 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.