INTERNATIONAL STANDARD

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Third edition
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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

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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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).

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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 6647-1:2015), 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.

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.