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Rice — Determination of amylose content —

Part 1: Reference method

Riz — Détermination de la teneur en amylose —

Partie 1: Méthode de référence

Reference number
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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 6647-1 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

This edition of ISO 6647-1, together with ISO 6647-2:2007, cancels and replaces ISO 6647:1987, which has been technically revised.

ISO 6647 consists of the following parts, under the general title *Rice — Determination of amylose content*:

— *Part 1: Reference method*

— *Part 2: Routine methods*

Rice — Determination of amylose content —

Part 1: Reference method

1 Scope

This part of ISO 6647 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 part of ISO 6647 can also be used for husked rice, maize, millet and other cereals if the extension of this scope has been validated by the user.

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 — Routine reference method*

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*

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

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 7301 and the following apply.

3.1
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 glucose units linked 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, to an aliquot portion of 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.

NOTE The method actually determines the affinity of amylose for iodine. The determination is made at 720 nm to minimize interfering effects of amylopectin.

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 dispersal solutions.

5.3.1 Sodium hydroxide, 1 mol/l solution.

5.3.2 Sodium hydroxide, 0,09 mol/l solution.

5.4 Deproteination solutions

5.4.1 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.

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

5.5 Acetic acid, 1 mol/l solution.

5.6 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.

5.7 Standard potato amylose suspension, free of amylopectin, 1 g/l.

5.7.1 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.

5.7.2 Spread the defatted potato amylose on a tray and leave for 2 days to allow evaporation of residual methanol and for moisture content equilibrium to be reached.

Treat the amylopectin (5.8) and the test samples (8.1) in the same way.

5.7.3 Weigh (6.9) 100 mg ± 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 (5.3.1) 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 standard suspension contains 1 mg of potato amylose.

When 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 standards are not prepared under the same conditions, the moisture content of both the samples and the standards has to be determined as specified in ISO 712 and the results should be corrected accordingly.

5.8 Standard amylopectin suspension, 1 g/l.

Prepare 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 detergent solution (5.4.1), or, alternatively, with sodium hydroxide solution (5.4.2), wash and then defat by refluxing with methanol (5.1) as described in 5.7.1. Spread the deproteinated and defatted amylopectin on a tray and leave for 2 days to allow evaporation of residual methanol and for moisture content equilibrium to be reached.

Carry out the procedure given in 5.7.3, but with amylopectin instead of amylose.

1 ml of this standard suspension contains 1 mg of amylopectin.

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

6 Apparatus

The usual laboratory apparatus and, in particular, the following.

6.1 Laboratory blender.

6.2 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.

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

6.4 Spectrophotometer, with matching cells, usually of pathlength 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 part of ISO 6647. A recommended sampling method is given in ISO 13690[3].

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 which will pass through the sieve (6.3).

Defat the flour by refluxing with methanol (5.1). Follow the procedure described in 5.7.1.

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. Higher amylose mass fractions will be obtained using defatted samples.

After defatting, spread the flour in a thin layer in a dish or watch glass and leave for 2 days to allow evaporation of residual methanol and for moisture content equilibrium to be reached (see 5.7).

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 ± 0,5 mg of the test sample (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. Add 9,0 ml of 1 mol/l sodium hydroxide solution (5.3.1) 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 (5.3.2) 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 (5.7) and amylopectin (5.8) standard suspensions and of the 0,09 mol/l sodium hydroxide solution (5.3.2) in accordance with Table 1.

Table 1 — Set of calibration solutions

Amylose mass fraction in milled rice, %, dry matter basis [a]	Potato amylose (5.7) ml	Amylopectin (5.8) ml	0,09 mol/l sodium hydroxide (5.3.2) 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

[a] 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 (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 (5.5) and mix. Then add 2,0 ml of iodine solution (5.6), 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 (8.3) 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 (8.2) into a 100 ml volumetric flask (6.6) containing about 50 ml of water and proceed according to 8.4.2, starting with the addition of acetic acid (5.5).

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

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

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, is obtained by referring the absorbance (8.5) to the calibration graph (8.4.3) according to ISO 8466-1.

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