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Rice — Determination of amylose content

Riz — Détermination de la teneur en amylose

Reference number
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Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 6647 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

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Rice — Determination of amylose content

1 Scope and field of application

This International Standard specifies a method for the determination of the amylose content of uncooked, milled rice.

2 Reference

ISO 950, *Cereals — Sampling (as grain).*

3 Definitions

For the purpose of this International Standard, the following definitions apply.

3.1 amylose: Polysaccharide constituent of starch, the macromolecules of which have a predominantly linear structure.

3.2 amylopectin: Polysaccharide constituent of starch, the macromolecules of which have a branched structure.

3.3 milled rice: Rice obtained after a milling operation which involves removing from the husked rice all or part of its pericarp and germ.

4 Principle

Grinding of the rice to a fine flour to destroy the crystallinity of the starch in order to aid complete dispersion and gelatinization, followed by defatting of the flour. Dispersion of a test portion in sodium hydroxide solution, then addition of an iodine solution to an aliquot portion and spectrometric determination of the absorbance, at 620 nm, of the colour complex formed.

Reading of the amylose content of the sample from a calibration graph, prepared using mixtures of 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

All the reagents used shall be of recognized analytical quality and the water used shall be distilled water or water of at least equivalent purity.

5.1 Methanol, 85 % (*V*/*V*).

5.2 Ethanol, 95 % (*V*/*V*).

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

5.4 Sodium hydroxide, 0,09 mol/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, make up to volume with water and mix.

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

5.7 Potato amylose, free of amylopectin, standard 1 g/l suspension.

5.7.1 Defat the amylose by refluxing with methanol (5.1) for 16 h in a Soxhlet extractor or for 4 h in a Goldfisch extractor at a rate of 5 to 6 drops/s (see note 1).

5.7.2 Spread the defatted 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) similarly (see note 2).

5.7.3 Weigh 100 ± 0,5 mg of the defatted and conditioned amylose into a 100 ml volumetric flask. Carefully add 1 ml of ethanol (5.2), rinsing down any amylose adhering to the walls of the flask. Add 9,0 ml of 1 mol/l sodium hydroxide solution (5.3) and leave at ambient temperature for 15 to 24 h, without shaking to disperse the amylose.

Make up to volume with water and mix vigorously.

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

NOTES

1 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 content of rice samples. Pure amylose should bind 19 % to 20 % of its own mass of iodine.

2 Because 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.

5.8 Amylopectin, standard 1 g/l suspension.

Prepare from milled glutinous (waxy) rice with a starch content known to be at least 99 % (*m/m*) 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 (sodium dodecylbenzene sulfonate, 20 g/l solution, with 2 g/l sodium sulfite solution added just before use) or alkali (sodium hydroxide, 3 g/l solution), wash and then defat by refluxing with methanol (5.1) for 16 h in a Soxhlet extractor or for 4 h in a Goldfisch extractor at a rate of 5 to 6 drops/s. 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. Treat the amylose (5.7) and the test samples (8.1) similarly (see note 2 to 5.7).

Carry out the procedure given in 5.7.3.

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

6 Apparatus

Usual laboratory equipment, and in particular

6.1 Laboratory blender, capable of reducing wet, steeped, milled rice to a finely divided state such that the resultant flour, when dried, will pass through a 250 µm mesh sieve.

6.2 Micro-mill, capable of reducing uncooked milled rice to a flour which will pass through a 250 µm mesh sieve, for example a cyclone mill or high-speed ball mill.

6.3 Sieve, of mesh size 250 µm.

6.4 Spectrometer, with matching cells, capable of measuring absorbance at 620 nm.

6.5 Extraction apparatus, Soxhlet or Goldfisch.

6.6 Volumetric flasks, 100 ml.

7 Sampling

See ISO 950.

8 Procedure

8.1 Preparation of test sample

In the micro-mill (6.2) grind at least 20 grains of milled rice to a very fine flour which will pass through the sieve (6.3). (See note 1.)

Defat the flour by refluxing with methanol (5.1) for 16 h in a Soxhlet extractor or for 4 h in a Goldfisch extractor at a rate of 5 to 6 drops/s. (See note 2.)

After defatting, spread the flour in a thin layer on 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 note 2 to 5.7).

NOTES

1 If it is desired to use a larger test sample which is beyond the capacity of the micro-mill, divide the sample into portions of suitable size and, after grinding each, thoroughly mix the portions of flour obtained. Alternatively the larger sample could be ground in a macro-mill capable of providing a homogeneous flour of the particle size required.

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

3 Results obtained by defatting with 85 % (*V/V*) methanol give values approximately 0,5 % less than those obtained when defatting is carried out using butanol, but this latter procedure is more difficult to apply.

8.2 Test portion

Weigh 100 ± 0,5 mg of the test sample into a 100 ml volumetric flask.

8.3 Preparation of the test solution

To the test portion (8.2), carefully add 1 ml of ethanol (5.2) using a pipette, washing down any of the test portion adhering to the side of the flask. Add 9,0 ml of 1 mol/l sodium hydroxide solution (5.3) from a pipette.

Allow to stand at room temperature for 15 to 24 h without shaking to disperse the starch. Alternatively, heat the test solution in a water-bath, brought to boiling point, for 10 min and then cool to room temperature.

Make up to volume with water and mix vigorously.

8.4 Blank test

Carry out a blank test in parallel with the determination, by the same procedure, using 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.

8.5 Preparation of the calibration graph

8.5.1 Preparation of the set of calibration solutions

Mix volumes of the amylose (5.7) and amylopectin (5.8) standard suspensions and of the 0,09 mol/l sodium hydroxide solution (5.4) in accordance with table 1.

NOTE — For routine analysis, defatted milled rice flours of predetermined amylose content may be used for the calibration in place of amylose and amylopectin suspensions.

8.5.2 Colour development

Pipette a 5,0 ml aliquot of each calibration solution into a series of five 100 ml one-mark volumetric flasks 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 20 min.

Table 1

Amylose in milled rice (m/m) % dry basis[1]	Composition of mixture (ml)		
	Amylose (5.7)	Amylopectin (5.8)	0,09 mol/l NaOH (5.4)
0	0	18	2
10	2	16	2
20	4	14	2
25	5	13	2
30	6	12	2

1) These values have been calculated on the basis of an average starch content of 90 % (m/m) in milled rice on the dry basis.

8.5.3 Spectrometric measurements

Measure the absorbance at 620 nm against the blank (8.4), using the spectrometer (6.3).

8.5.4 Plotting the calibration graph

Prepare a calibration graph by plotting absorbance against the amylose content, expressed as a percentage by mass, in the milled rice on the dry basis.

8.6 Determination

8.6.1 Colour development

Pipette a 5,0 ml aliquot of the test solution (8.3) into a 100 ml one-mark volumetric flask containing about 50 ml of water, and proceed according to 8.5.2, starting with the addition of acetic acid.

8.6.2 Spectrometric measurements

Measure the absorbance at 620 nm against the blank (8.4), using the spectrometer (6.4).

8.7 Number of determinations

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

9 Expression of results

The amylose content, expressed as a percentage by mass on a dry basis, is obtained by referring the absorbance (8.6.2) to the calibration graph (8.5.4).

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

10 Precision

An international inter-laboratory study carried out by the International Rice Research Institute (Manila, Philippines) with the participation of nine laboratories, each of which carried out two determinations, has given the statistical results (analysed in accordance with ISO 5725[1]) shown in table 2.

11 Test report

The test report shall show the method used and the result obtained. It shall also mention any operating details not specified in this International Standard, or regarded as optional, together with details at any incidents likely to have influenced the result.

The test report shall include all the information necessary for the complete identification of the sample.

1) ISO 5725, *Precision of test methods — Determination of repeatability and reproducibility by inter-laboratory tests.*

Table 2

Sample	IR 2071-137-5	IR 3351-38-03	C 4-63 G	IR 8	IR 5
Number of laboratories remaining after elimination of outliers	9	8	9	9	9
Mean	8,5	11,0	19,8	24,2	24,9
Standard deviation of a repeatability (S_r) Coefficient of variation of repeatability Repeatability (2,83 × S_r)	0,24 2,8 % 0,7	0,36 3,3 % 1,0	0,43 2,2 % 1,2	0,38 1,6 % 1,1	0,38 1,5 % 1,1
Standard deviation of reproducibility (S_R) Coefficient of variation of reproducibility Reproducibility (2,83 × S_R)	0,9 10,3 % 2,5	0,6 5,2 % 1,6	1,3 6,5 % 3,6	0,8 3,4 % 2,3	1,3 5,4 % 3,8

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