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**Rice — Determination of amylose  
content —**

**Part 2:  
Routine methods**

*Riz — Détermination de la teneur en amylose —  
Partie 2: Méthodes de routine*



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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.

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 6647-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

This edition of ISO 6647-2, together with ISO 6647-1:2007, 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 2: Routine methods

### 1 Scope

This part of ISO 6647 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, and method B 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 of which the amylose mass fraction has been determined by the reference method are used as standards.

### 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 6647-1, *Rice — Determination of amylose content — Part 1: 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*

### 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 6647-1 and ISO 7301 apply.

### 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, to an aliquot portion of 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 in ISO 6647-1.

Standard rice samples are used to circumvent the interference from fat on the colour reaction, without defatting the samples and standards. Both samples and standard samples should be well milled to minimize the lipid interference.

## 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**

**5.2.1 1 mol/l solution**, for method A.

**5.2.2 2 mol/l solution**, for method B.

**5.3 Sodium hydroxide**

**5.3.1 0,09 mol/l solution**, for method A.

**5.3.2 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

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

**6.1 Grinder**, capable of reducing uncooked milled rice to a fine 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.

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

**6.3 Spectrophotometer**, with matching cells, usually of pathlength 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.

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

**6.9 Pipettes**, of capacity 1 ml, 2 ml, 5 ml and 10 ml.

## 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<sup>[3]</sup>.

## 8 Procedure

### 8.1 Determination of moisture

On a separate portion of the laboratory sample and the standard samples, carry out a moisture determination in accordance with ISO 712.

### 8.2 Preparation of test sample

In the grinder (6.1) grind at least 10 g of milled rice which will pass through the sieve (6.2).

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

**8.3.1** Weigh (6.8) 100 mg  $\pm$  0,5 mg of the test sample (8.2) into a 100 ml conical flask (6.7). To this test portion carefully add 1 ml of ethanol (5.1) using a pipette, washing down any of the test portion adhering to the side of the flask. Shake slightly in order to wet the entire sample.

#### 8.3.2 Method A

Pipette (6.9) 9,0 ml of sodium hydroxide solution (5.2.1) into the conical flask and mix. Then heat the mixture on a boiling water bath (6.5) for 10 min to disperse the starch. Allow to cool to room temperature and transfer quantitatively to a 100 ml volumetric flask (6.4). Make up to volume with water and mix vigorously.

#### 8.3.3 Method B

Pipette (6.9) 9,0 ml of sodium hydroxide solution (5.2.2) into the conical flask and mix. Stir the mixture using a magnetic stirrer (6.6) for 10 min to obtain the dispersion. Remove the stirrer and transfer quantitatively to a 100 ml volumetric flask (6.4). Make up to volume with water and mix vigorously.

It is recommended to swirl the liquid in the volumetric flask before adding the water and after making up to volume.

### 8.4 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 sodium hydroxide solution (5.3.1 for method A and 5.3.2 for method B) instead of the test solution.

## 8.5 Preparation of the calibration graph

### 8.5.1 Preparation of the set of calibration solutions

Select at least four rice samples with a distribution of amylose mass fraction in the measured range. For each sample, ensure that the amylose mass fraction has been determined by the reference method specified in ISO 6647-1 independently, 20 times.

Alternatively, a certified reference material may be used.

Prepare the calibration solutions as in 8.2 and 8.3.

### 8.5.2 Colour development and spectrophotometric measurements

Pipette (6.9) a 5,0 ml aliquot of each calibration solution into a series of five volumetric flasks (6.4) each containing about 50 ml of water. Pipette (6.9) 1,0 ml of acetic acid (5.4) for method A or 2,0 ml for method B and mix. Then pipette (6.9) 2,0 ml of iodine solution (5.5), 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 (8.4) using the spectrophotometer (6.3).

Measurement wavelengths of 620 nm or 680 nm can also be used (see Annex A).

### 8.5.3 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.

Instead of manual spectrometric measurements, an automatic analyser, e.g. a flow injection analyser, may be used (see ISO 6647-1:2007, Annex B).

## 8.6 Determination

Pipette (6.9) a 5,0 ml aliquot of the test solution (8.3) into a volumetric flask (6.4) containing about 50 ml of water and proceed according to 8.5.2, starting with the addition of acetic acid (5.4).

Measure the absorbance at 720 nm (or at 620 nm or at 680 nm, see Annex A) against the blank solution (8.4) using the spectrophotometer (6.3).

Instead of manual spectrometric measurements, an automatic analyser, e.g. a flow injection analyser, may be used (see ISO 6647-1:2007, Annex B).

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

If double determinations are made, based on two independent preparations of the sample (8.2), this should be noted in the test report.

## 9 Expression of results

The amylose mass fraction, expressed as a percentage on the dry basis, is obtained by referring the absorbance (8.6) to the calibration graph (8.5.3) in accordance with ISO 8466-1.

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

Any result given should clearly refer to the method used (i.e. whether, for calibration, amylose solutions or rice samples analysed in accordance with ISO 6647-1 have been used).



## 10 Precision

### 10.1 Interlaboratory test

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

### 10.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time will in not more that 5 % of cases exceed the repeatability limit,  $r_{720}$ , expressed as a percentage by mass, calculated from the following equations:

Method A:

$$r_{720} = 22,47 \times \frac{1}{\bar{w}^{0,66}}$$

Method B:

$$r_{720} = 24,01 \times \frac{1}{\bar{w}^{0,61}}$$

where

$\bar{w}$  is the mean of the two mass fraction results, expressed in grams per 100 g;

720 is the wavelength, in nanometres, at which the absorbance was measured.

### 10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment will in not more than 5 % of cases be greater than the reproducibility limit,  $R_{720}$ , expressed as a percentage by mass, calculated from the following equations:

Method A:

$$R_{720} = 50,55 \times \frac{1}{\bar{w}^{0,68}}$$

Method B:

$$R_{720} = 83,11 \times \frac{1}{\bar{w}^{0,63}}$$

where

$\bar{w}$  is the mean of the two mass fraction results, expressed in grams per 100 g;

720 is the wavelength, in nanometres, at which the absorbance was measured.

## 11 Test report

The test report shall specify the following:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method used (A or B), with reference to this part of ISO 6647;
- d) all operating details not specified in this part of ISO 6647, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- e) the test result(s) obtained, or, if the repeatability has been checked, the final quoted result obtained.

## Annex A (informative)

### Results of an interlaboratory test

An interlaboratory test organized by FOSS Analytical AB (Sweden) in 2004, involving 23 laboratories in 11 countries including two international organizations, was carried out on six rice samples containing amylose at various mass fractions, which were provided by the Thai Industrial Standards Institute.

The results obtained were subjected to statistical analysis, which was performed by the Hungarian Standards Institution, in accordance with ISO 5725-1<sup>[1]</sup> and ISO 5725-2<sup>[2]</sup> to give the precision data shown in Tables A.1 to A.6.

#### A.1 Interlaboratory test results at 720 nm

**Table A.1 — Results of the statistical evaluation for method A (hot dispersion)**

	Rice samples					
	A	B	C	D	E	F
Number of laboratories after eliminating outliers	21	21	22	22	22	19
Mean mass fraction, g/100 g	10,79	23,73	12,84	25,74	2,28	27,77
Repeatability standard deviation, $s_r$ , g/100 g	0,52	0,67	0,51	0,81	0,30	0,60
Coefficient of variation of repeatability, %	4,82	2,84	3,98	3,14	13,06	2,16
Repeatability limit, $r = 2,8s_r$ , g/100 g	1,46	1,89	1,43	2,27	0,84	1,68
Reproducibility standard deviation, $s_R$ , g/100 g	1,07	1,54	1,15	1,48	0,66	1,33
Coefficient of variation of reproducibility, %	9,95	6,47	8,93	5,75	28,86	4,80
Reproducibility limit, $R = 2,8s_R$ , g/100 g	3,01	4,30	3,21	4,14	1,85	3,73

**Table A.2 — Results of the statistical evaluation for method B (cold dispersion)**

	Rice samples					
	A	B	C	D	E	F
Number of laboratories after eliminating outliers	20	20	18	20	21	20
Mean mass fraction, g/100 g	10,99	23,48	13,35	26,15	1,98	27,89
Repeatability standard deviation, $s_r$ , g/100 g	0,50	0,97	0,53	0,87	0,35	0,99
Coefficient of variation of repeatability, %	4,58	4,15	3,95	3,33	17,83	3,54
Repeatability limit, $r = 2,8s_r$ , g/100 g	1,41	2,73	1,48	2,44	0,99	2,76
Reproducibility standard deviation, $s_R$ , g/100 g	1,16	1,54	0,85	1,96	1,06	1,44
Coefficient of variation of reproducibility, %	10,55	6,57	6,40	7,50	53,70	5,18
Reproducibility limit, $R = 2,8s_R$ , g/100 g	3,25	4,32	2,39	5,49	2,98	4,04

## A.2 Interlaboratory test results at 680 nm

Table A.3 — Results of the statistical evaluation for method A (hot dispersion)

	Rice samples					
	A	B	C	D	E	F
Number of laboratories after eliminating outliers	21	20	21	20	22	18
Mean mass fraction, g/100 g	11,31	23,71	13,28	25,83	2,38	27,69
Repeatability standard deviation, $s_r$ , g/100 g	0,60	0,68	0,49	0,75	0,29	0,59
Coefficient of variation of repeatability, %	5,31	2,86	3,70	2,89	12,19	2,13
Repeatability limit, $r = 2,8s_r$ , g/100 g	1,68	1,90	1,38	2,09	0,81	1,65
Reproducibility standard deviation, $s_R$ , g/100 g	1,12	1,32	1,17	1,35	0,81	1,25
Coefficient of variation of reproducibility, %	9,94	5,58	8,78	5,22	34,11	4,53
Reproducibility limit, $R = 2,8s_R$ , g/100 g	3,15	3,70	3,26	3,77	2,27	3,51

Table A.4 — Results of the statistical evaluation for method B (cold dispersion)

	Rice samples					
	A	B	C	D	E	F
Number of laboratories after eliminating outliers	18	16	18	18	19	19
Mean mass fraction, g/100 g	11,48	23,66	13,65	26,39	1,97	27,87
Repeatability standard deviation, $s_r$ , g/100 g	0,47	0,52	0,56	0,68	0,37	0,80
Coefficient of variation of repeatability, %	4,08	2,22	4,08	2,58	18,95	2,86
Repeatability limit, $r = 2,8s_r$ , g/100 g	1,31	1,47	1,56	1,91	1,05	2,23
Reproducibility standard deviation, $s_R$ , g/100 g	0,92	1,12	0,86	1,44	1,00	1,42
Coefficient of variation of reproducibility, %	8,01	4,72	6,32	5,44	50,69	5,10
Reproducibility limit, $R = 2,8s_R$ , g/100 g	2,57	3,13	2,42	4,02	2,80	3,98

## A.2.1 Repeatability of the methods

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time will in not more that 5 % of cases exceed the repeatability limit,  $r_{680}$ , expressed as a percentage by mass, calculated from the following equations:

Method A:

$$r_{680} = 22,39 \times \frac{1}{\bar{w} \cdot 0,66}$$

Method B:

$$r_{680} = 30,33 \times \frac{1}{\bar{w} 0,77}$$

where

$\bar{w}$  is the mean of the two mass fraction results, expressed in grams per 100 g;

680 is the wavelength, in nanometres, at which the absorbance was measured.

### A.2.2 Reproducibility of the methods

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment will in not more than 5 % of cases be greater than the reproducibility limit,  $R_{680}$ , expressed as a percentage by mass, calculated from the following equations:

Method A:

$$R_{680} = 68,97 \times \frac{1}{\bar{w} 0,80}$$

Method B:

$$R_{680} = 81,63 \times \frac{1}{\bar{w} 0,88}$$

where

$\bar{w}$  is the mean of the two mass fraction results, expressed in grams per 100 g;

680 is the wavelength, in nanometres, at which the absorbance was measured.

### A.3 Interlaboratory test results at 620 nm

Table A.5 — Results of the statistical evaluation for method A (hot dispersion)

	Rice samples					
	A	B	C	D	E	F
Number of laboratories after eliminating outliers	21	23	22	21	22	21
Mean mass fraction, g/100 g	11,30	23,43	13,20	25,43	2,18	27,65
Repeatability standard deviation, $s_p$ , g/100 g	0,55	0,76	0,46	0,78	0,42	0,96
Coefficient of variation of repeatability, %	4,68	3,23	3,39	3,08	19,38	3,55
Repeatability limit, $r = 2,8s_p$ , g/100 g	1,55	2,13	1,30	2,20	1,18	2,70
Reproducibility standard deviation, $s_R$ , g/100 g	1,05	1,56	1,30	1,47	1,06	1,45
Coefficient of variation of reproducibility, %	8,87	6,61	9,53	5,76	48,45	5,36
Reproducibility limit, $R = 2,8s_R$ , g/100 g	2,94	4,35	3,65	4,11	2,96	4,07

**Table A.6 — Results of the statistical evaluation for method B (cold dispersion)**

	Rice samples					
	A	B	C	D	E	F
Number of laboratories after eliminating outliers	18	20	17	18	19	19
Mean mass fraction, g/100 g	12,45	24,08	14,09	26,35	1,79	27,92
Repeatability standard deviation, $s_r$ , g/100 g	0,51	1,02	0,47	0,56	0,47	0,72
Coefficient of variation of repeatability, %	4,06	4,22	3,31	2,11	26,42	2,57
Repeatability limit, $r = 2,8s_r$ , g/100 g	1,42	2,85	1,31	1,56	1,32	2,01
Reproducibility standard deviation, $s_R$ , g/100 g	1,22	1,97	0,90	1,66	1,29	1,76
Coefficient of variation of reproducibility, %	9,76	8,17	6,37	6,30	72,29	6,29
Reproducibility limit, $R = 2,8s_R$ , g/100 g	3,40	5,51	2,51	4,65	3,62	4,92

**A.3.1 Repeatability of the methods**

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time will in not more that 5 % of cases exceed the repeatability limit,  $r_{620}$ , expressed as a percentage by mass, calculated from the following equations:

Method A:

$$r_{620} = 29,46 \times \frac{1}{\bar{w}^{0,71}}$$

Method B:

$$r_{620} = 39,30 \times \frac{1}{\bar{w}^{0,84}}$$

where

$\bar{w}$  is the mean of the two mass fraction results, expressed in grams per 100 g;

620 is the wavelength, in nanometres, at which the absorbance was measured.

**A.3.2 Reproducibility of the methods**

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment will in not more than 5 % of cases be greater than the reproducibility limit,  $R_{620}$ , expressed as a percentage by mass, calculated from the following equations:

Method A:

$$R_{620} = 86,77 \times \frac{1}{\bar{w}^{0,85}}$$

Method B:

$$R_{620} = 104,68 \times \frac{1}{\bar{w} \cdot 0,88}$$

where

$\bar{w}$  is the mean of the two mass fraction results, expressed in grams per 100 g;

620 is the wavelength, in nanometres, at which the absorbance was measured.

## Bibliography

- [1] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*
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- [3] ISO 13690:1999, *Cereals, pulses and milled products — Sampling of static batches*
- [4] AACC method 61-03 (re-approved 1999; this method is equivalent to the withdrawn ISO 6647:1987)
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- [6] JULIANO B.O. A simplified assay for milled rice amylose. *Cereal Sci. Today*, 1971, **16**, pp. 334–40, 360





