
Wheat — Determination of the sedimentation index — Zeleny test

*Blé tendre — Détermination de l'indice de sédimentation —
Test de Zélény*

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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 5529 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

This third edition cancels and replaces the second edition (ISO 5529:1992), which has been technically revised.

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Wheat — Determination of the sedimentation index — Zeleny test

1 Scope

This International Standard describes a method, known as the Zeleny sedimentation test, for assessing one of the factors determining the quality of wheat as a means of predicting the baking strength of the flour which can be made from it.

The method is applicable only to *Triticum aestivum* L. wheat.

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 565, *Test sieves — Metal wire cloth, perforated metal plate and electroformed sheet — Nominal sizes of openings*

ISO 648, *Laboratory glassware — One-mark pipettes*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

sedimentation index

number indicating the volume of the sediment obtained, under the conditions specified in this International Standard, from a suspension of test flour, prepared from the wheat, in a solution of lactic acid and propan-2-ol

NOTE 1 The sedimentation index is determined by the Zeleny test.

NOTE 2 The volume is expressed in millilitres.

4 Principle

The measurement principle is based on the ability of the flour proteins to swell in an acid medium.

Test flour, prepared from wheat under specified grinding and sieving conditions, is suspended in a solution of lactic acid and propan-2-ol in the presence of a dye. After specified shaking and rest times, the obtained sediment volume corresponds to the sedimentation of the flour particles.

5 Reagents

Use reagents of recognized analytical grade, unless otherwise specified, and water complying with grade 2 in accordance with ISO 3696, unless otherwise stated.

5.1 Reagents for the sedimentation test

5.1.1 Lactic (2-hydroxypropanoic) **acid solution**, at an aqueous volume fraction of 90 %, $M = 90,08$ g/mol, $d = 1,20$ to $1,22$.

5.1.2 Propan-2-ol, at a volume fraction of 99 % to 100 %, $M = 60,10$ g/mol.

5.1.3 Sodium hydroxide, standard solution, $\rho(\text{NaOH}) = 40$ g/l.

5.1.4 Bromophenol blue, $\text{C}_{19}\text{H}_{10}\text{Br}_4\text{O}_5\text{S}$, solution.

In a 1 000 ml volumetric flask (6.6), dissolve 4 mg of bromophenol blue in water, then make up to the mark with water.

5.1.5 Phenolphthalein, $\text{C}_{20}\text{H}_{14}\text{O}_4$, solution.

In a 100 ml volumetric flask (6.7), dissolve 1 g of phenolphthalein in ethanol at an aqueous volume fraction of 95 % to 96 %, then make up to the mark with ethanol.

5.2 Preparation of the solutions

5.2.1 Lactic acid stock solution

Pour 235 ml of lactic acid solution (5.1.1) into a 1 000 ml volumetric flask (6.6) and adjust to the mark with water. Transfer the solution to the flask (6.8) and place the latter on the heating mantle (6.9). Bring to the boil and reflux for 6 h.

Concentrated lactic acid solution contains associated molecules which, on dilution, dissociate slowly to equilibrium. Boiling accelerates this dissociation process which is essential in order to obtain reproducible sedimentation values.

Leave to cool for at least 2 h prior to titration. Then titrate (6.12) 10 ml of this solution against sodium hydroxide (5.1.3) using phenolphthalein (5.1.5) as indicator (10 ml of lactic acid solution require about 28 ml of sodium hydroxide). The concentration found shall be between 2,7 mol/l and 2,8 mol/l.

Store the lactic acid stock solution in a tinted glass bottle.

5.2.2 Test solution

In a 1 000 ml volumetric flask (6.6), mix 180 ml of the lactic acid stock solution (5.2.1) and 200 ml of propan-2-ol (5.1.2), then make up to the mark with water.

Store in a stoppered bottle. After preparation, only use the reagent after it has stood for a minimum of 48 h and within a maximum period of 15 days.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Test mill¹⁾ (see Annex A).

6.2 Woven metal wire sieve²⁾, in accordance with the specifications of ISO 565, of nominal aperture size 150 μm , of diameter 200 mm, moved by an appropriate automatic vibration device of eccentricity 50 mm and rotational frequency 200 min^{-1} .

6.3 Laboratory sample divider.

6.4 Flat-bottom cylinders, of capacity 100 ml, graduated in millilitres, with a distance of 180 mm to 185 mm between the bottom and the 100 ml mark, and equipped with plastic or glass stoppers.

6.5 Cylinder shaker, fitted with a time switch and providing a shaking frequency of 40 min^{-1} ; each cycle shall be through 60° (30° above and below the horizontal).

6.6 One-mark volumetric flasks, of capacity 1 000 ml.

6.7 One-mark volumetric flask, of capacity 100 ml.

6.8 Flask, of capacity 1 500 ml, **equipped with a reflux condenser**.

6.9 Heating mantle.

6.10 One-mark pipettes, of capacity 25 ml and 50 ml, conforming to ISO 648, or **automatic dispensers**.

6.11 Analytical balance, capable of weighing to the nearest 0,01 g, with a readability of 0,001 g.

6.12 Equipment used for the titration of the lactic acid stock solution (5.2.1).

6.12.1 Two-mark pipette, of capacity 10 ml.

6.12.2 Burette, of capacity 50 ml, graduated in 0,1 ml increments.

6.12.3 Beaker, of capacity 50 ml.

6.12.4 Magnetic stirrer and stirrer bar.

6.13 Stopwatch.

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 International Standard. A recommended sampling method is given in ISO 13690.

1) The Miag-Grobschrotmühle; Brabender-Sedimat; Strand-Roll, model SRM; Straube, model W.1; and Tag-Heppenstall are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

2) The Brabender-Sedimat mill incorporates a sieving device (see A.3).

8 Preparation of the test flour

Discard all the impurities from the wheat, either manually or using a mechanical grain cleaning machine.

Mix, then divide the laboratory sample using a divider (6.3) until a mass corresponding to the type of mill (6.1) used for grinding (see Annex A) is obtained. Grind the grain and sieve the ground product as described in Annex A.

Repeat this operation to obtain a second test sample.

Grinding conditions differ according to the type of mill, but in all cases the particle size of the test flour shall be $\leq 150 \mu\text{m}$ and the extracted mass $\geq 10\%$ of the mass of wheat used.

9 Procedure

9.1 Test portion

Mix both test flour samples (Clause 8). Weigh (6.11), to the nearest 0,05 g, two test portions of 3,2 g from each of the test samples.

NOTE If there is any reason to think that the moisture content of the test flour is outside the range 13 % to 15 %, determine its value in accordance with ISO 712, then weigh a quantity of test flour corresponding to $3,20 \text{ g} \pm 0,05 \text{ g}$ at a moisture mass fraction of 14 % (i.e. $2,75 \text{ g} \pm 0,04 \text{ g}$ of dry matter).

9.2 Determination

Carry out the tests under normal lighting conditions, out of direct sunlight.

Place the test portion (9.1) in a measuring cylinder (6.4).

Using a pipette or an automatic dispenser (6.10), add $(50 \pm 0,5)$ ml of the bromophenol blue solution (5.1.4) to the test portion. Stopper the cylinder, and maintaining it in a horizontal position, shake it longitudinally from right to left, through approximately 18 cm to 20 cm, 12 times in each direction within approximately 5 s.

Place the cylinder in the shaker (6.5), then start the stopwatch (6.13) and the shaker. After 5 min, remove the cylinder from the shaker and using a pipette or the automatic dispenser (6.10) add to its contents $25 \text{ ml} \pm 0,2 \text{ ml}$ of the test solution (5.2.2).

Replace the cylinder in the shaker and continue shaking for 5 min to give a total shaking time of 10 min.

Remove the cylinder and place it in an upright position.

Leave the contents of the cylinder to stand for $300 \text{ s} \pm 5 \text{ s}$, then note the volume of the deposit to the nearest 1 ml.

10 Expression of results

The number indicating the volume, expressed in millilitres, of the deposit noted in 9.2 represents the sedimentation index.

Take as the result the arithmetic mean of the results if the limit of repeatability is correct. If the difference exceeds the repeatability limit, r (11.2), repeat the operations described in 9.1 and 9.2. If the difference between the two new determinations does not exceed the repeatability limit, take the arithmetic mean of the two new determinations and discard the two first results. In the opposite case, take the arithmetic mean of the four individual determinations.

Express the result as a whole number.

11 Precision

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex B. The values derived from this interlaboratory test cannot be applied to other matrix and concentration ranges than those given.

11.2 Repeatability limit

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 than 5 % of cases exceed the repeatability limit, r , calculated from the following equation:

$$r = (0,009\,8\bar{V}_Z + 0,317\,9) \times 2,77$$

where $\bar{V}_Z$ is the mean of the two test results, expressed in millilitres.

11.3 Reproducibility limit

The absolute difference between two independent single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases exceed the reproducibility limit, R , calculated from the following equation:

$$R = (0,037\,2\bar{V}_Z + 0,266\,5) \times 2,77$$

where $\bar{V}_Z$ is the mean of the two test results, expressed in millilitres.

11.4 Critical difference

Critical difference represents the deviation between two averaged values obtained from two test results under repeatability conditions.

11.4.1 Comparison of two groups of measurements in one laboratory

The critical difference of repeatability, $CD(r)$, between two averages, each obtained from two test results under repeatability conditions, is calculated from Equation (1):

$$CD(r) = 2,8s_r \sqrt{\frac{1}{2n_1} + \frac{1}{2n_2}} = 2,8s_r / \sqrt{2} = 1,98s_r \quad (1)$$

where

s_r is the standard deviation of repeatability;

n_1 and n_2 are the number of test results corresponding to each of the averaged values.

11.4.2 Comparison of two groups of measurements in two laboratories

The critical difference of reproducibility, $CD(R)$, between two averages, each obtained from two test results by two different laboratories, each under repeatability conditions, is calculated from Equation (2):

$$CD(R) = 2,8 \sqrt{s_R^2 - s_r^2 \left(1 - \frac{1}{2n_1} - \frac{1}{2n_2}\right)} = 2,8 \sqrt{s_R^2 - 0,5s_r^2} \quad (2)$$

where

s_r is the standard deviation of repeatability;

s_R is the standard deviation of reproducibility;

n_1 and n_2 are the number of test results corresponding to each of the averaged values.

12 Test report

The test report shall specify:

- a) all information required for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the type of mill used;
- d) the test method used, together with a reference to this International Standard;
- e) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- f) the test result(s) obtained.

Annex A (normative)

Sample grinding and sieving conditions according to the different types of mills

A.1 General

The sample mass specified in A.2.1 to A.2.5, freed from impurities, shall be ground under the conditions specified below according to the type of mill being used.

A.2 Mill types

A.2.1 Miag-Grobschrotmühle mill³⁾

Sample: 100 g.

Carry out an initial grinding with a 1 mm roll gap and at a rotational frequency of about 30 min⁻¹. Regrind all of the material obtained from the initial grinding, but with a roll gap of 0,1 mm, then repeat this regrinding procedure with the same gap. Using the sieve (6.2), sieve the product obtained after these three successive grindings for 5 min.

A.2.2 Brabender-Sedimat mill³⁾

Sample: 100 g.

NOTE 1 This mill comprises grooved rolls, a revolving sieve having an aperture size of 150 µm and a timing device.

The grinding device is fitted with 4 grooved rolls with a diameter of 71 mm: one cylinder with 160 grooves; and three identical cylinders with grooves parallel to this axis. The roll gaps recommended by the manufacturer are: 0,8 mm to 1,0 mm between the first and the second cylinder; 0,1 mm between the second and the third cylinders; and 0,03 to 0,04 mm between the third and the fourth cylinders. Rotational speed is not adjustable. It is advisable to check the rolls for wear by determining that the gaps between each of the grinding rolls comply with the recommendations.

Set the mill timing device at 3 min 5 s. Start the mill, and after 5 s ensure that all the grain has been introduced into the hopper. Leave the mill to grind until the mill stops.

NOTE 2 Generally, grinding takes 1 min and sieving 2 min.

A.2.3 Tag-Heppenstall mill³⁾

Sample: 200 g.

Carry out an initial grinding with a 0,6 mm roll gap and at a rotational frequency of about 30 min⁻¹. Regrind all of the material obtained from the initial grinding with the same roll gap, then repeat this regrinding procedure a further three times. Sieve (6.2) the product obtained after these five successive grindings for 1 min 30 s.

3) Example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

A.2.4 Strand-Roll mill, Model SRM⁴⁾

Sample: 150 g.

Carry out an initial grinding with a 0,8 mm roll gap and at a rotational frequency of about 30 min⁻¹. Regrind all of the material obtained from the initial grinding with the same roll gap, then repeat this regrinding procedure a further three times. Sieve (6.2) the product obtained after these five successive grindings for 1 min 30 s.

A.2.5 Straube mill, model W.1⁴⁾

Sample: 150 g.

Proceed as in A.2.4, carrying out the five grindings with a 1,10 mm roll gap and at a rotational frequency of about 60 min⁻¹.

A.3 Cleaning

Between successive grinding and sieving operations with different samples of wheat, the mills and sieves shall be suitably cleaned.

4) Example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

Annex B (informative)

Interlaboratory test results

An international collaborative test involving 13 laboratories in 5 countries was carried out on 6 wheat samples in August 2003.

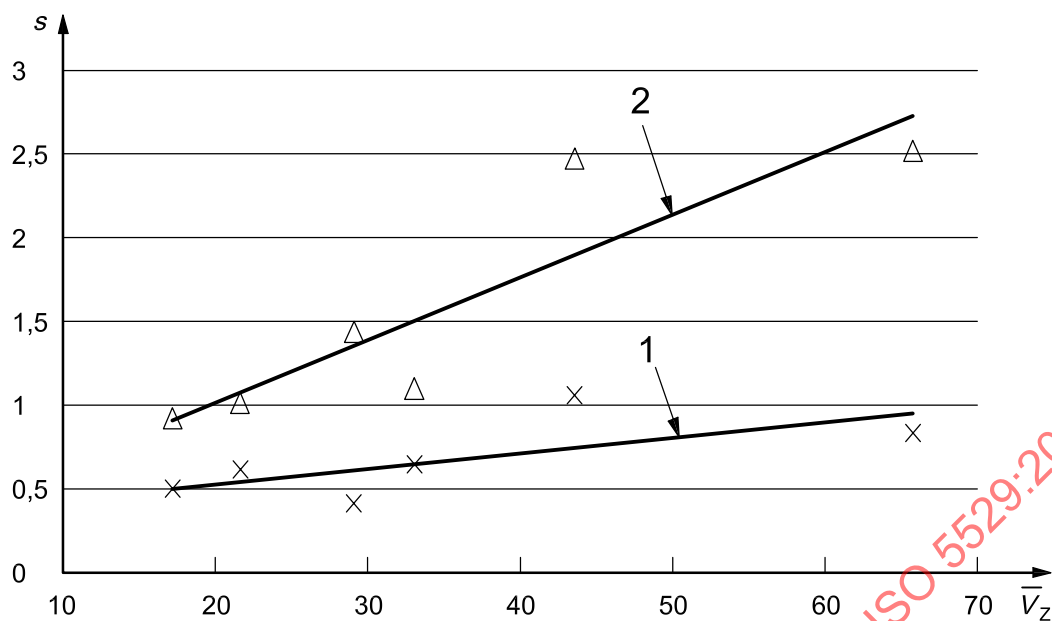
The results obtained were subjected to statistical analysis in accordance with ISO 5725-1, ISO 5725-2 and ISO 5725-6, and gave the precision data shown in Table B.1.

The design of this test allowed the variability between two test portions of 3,2 g from the same grinding and between two test portions of 3,2 g from two different 100 g grindings to be studied. In the first case, as the variance was equal to 0, repeatability parameters assessed by the study are to be considered as an intermediate reproducibility which can be considered as a repeatability if the two grindings are performed on the same day.

The calculated repeatability and reproducibility limits from precision data are listed in Table B.2.

Table B.1 — Statistical results of the interlaboratory test

Wheat sample	No. of labs after eliminating outliers	Mean value ml	Repeatability standard deviation s_r ml	Coefficient of variation of repeatability $CV(r)$ %	Repeatability limit $r = 2,8s_r$ ml	Reproducibility standard deviation s_R ml	Coefficient of variation of reproducibility $CV(R)$ %	Reproducibility limit $R = 2,8s_R$ ml
1	11	21,7	0,6	2,9	1,7	1,0	4,7	2,8
2	10	29,0	0,4	1,4	1,1	1,4	4,9	4,0
3	11	65,7	0,8	1,3	2,3	2,5	3,8	7,0
4	11	43,5	1,1	2,4	2,9	2,5	5,7	6,8
5	11	33,0	0,6	1,9	1,8	1,1	3,3	3,0
6	11	17,3	0,4	2,4	1,1	0,9	5,3	2,5



Key

1 repeatability regression line:
 $s_r = 0,009\ 8\ \bar{V}_Z + 0,317\ 9$
 (correlation coefficient, $R^2 = 0,5$)

2 reproducibility regression line:
 $s_R = 0,037\ 2\ \bar{V}_Z + 0,266\ 5$
 (correlation coefficient, $R^2 = 0,8$)

s standard deviation

$\bar{V}_Z$ mean Zeleny sedimentation index

x standard deviation of repeatability, s_r

Δ standard deviation of reproducibility, s_R

Figure B.1 — Relationship between precision standard deviations and mean values