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Wheat — Determination of the sedimentation index — Zeleny test

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

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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 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.

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, ρ(NaOH) = 40 g/l.

5.1.4 Bromophenol blue, $C_{19}H_{10}Br_4O_5S$, 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, $C_{20}H_{14}O_4$, 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 mill[1] (see Annex A).

6.2 Woven metal wire sieve[2], 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.

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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 $\leqslant$150 µm and the extracted mass $\geqslant$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 g ± 0,05 g at a moisture mass fraction of 14 % (i.e. 2,75 g ± 0,04 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 ± 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 ml ± 0,2 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 s ± 5 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):

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

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.