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ISO 3093

Fourth edition
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Wheat, rye and their flours, durum wheat and durum wheat semolina — Determination of the falling number according to Hagberg-Perten

Blés tendres, seigles et leurs farines, blés durs et leurs semoules — Détermination de l'indice de chute selon Hagberg-Perten

Reference number
ISO 3093:2009(E)

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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.

ISO draws attention to the fact that it is claimed that compliance with this document may involve the use of a patent concerning the falling number apparatus specified in 6.1.

ISO takes no position concerning the evidence, validity and scope of this patent right.

The holder of this patent right has assured the ISO that he is willing to negotiate licences under reasonable and non-discriminatory terms and conditions with applicants throughout the world. In this respect, the statement of the holder of this patent right is registered with the ISO. Information may be obtained from:

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Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights other than those identified above. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 3093 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

This fourth edition cancels and replaces the third edition (ISO 3093:2004), of which it constitutes a minor revision. It also incorporates the Technical Corrigendum, ISO 3093:2004/Cor.1:2008.

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Wheat, rye and their flours, durum wheat and durum wheat semolina — Determination of the falling number according to Hagberg-Perten

1 Scope

This International Standard specifies the determination of the α-amylase activity of cereals by the falling number (FN) method according to Hagberg-Perten.

This method is applicable to cereal grains, in particular to wheat and rye and their flours, durum wheat and its semolina.

This method is not applicable to the determination of low levels of α-amylase activity.

By converting the FN into a liquefaction number (LN), it is possible to use this method to estimate the composition of mixtures of grain, flour or semolina with known FNs necessary to produce a sample of a required FN.

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 — Reference method*

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
falling number
FN
t
total time required to activate a viscometer stirrer and allow it to fall a predetermined distance through an aqueous gel prepared from heating a mixture of flour or semolina, and water in a viscometer tube, and which is undergoing liquefaction due to attack by the enzyme α-amylase

NOTE 1 Time is counted from immersion in the water bath.

NOTE 2 The falling number is expressed in seconds.

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3.2
liquefaction number
LN
n_L
result of a simple calculation to convert the **falling number** (3.1) into a value used to estimate the composition of mixtures of grain, flour or semolina necessary to produce a sample of the required falling number

NOTE LNs, unlike FNs, are additive.

4 Principle

The α-amylase activity is estimated using the starch present in the sample as a substrate. The determination is based on the ability of an aqueous suspension of flour, semolina or wholemeal cereal product to gelatinize rapidly in a boiling water bath, and on the measurement of starch liquefaction by the α-amylase present in the sample.

Liquefaction affects the thickness of the starch gel and, hence, the resistance to the viscometer stirrer and the time taken for it to fall a defined distance.

5 Reagents

5.1 Water, produced by distillation or demineralization, complying with ISO 3696, grade 3.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Apparatus for FN determination[1)], comprising the following components.

6.1.1 Water bath, with integral heating unit, cooling system and water level indicator.

6.1.2 Electronic timer.

6.1.3 Viscometer stirrer, metallic, able to move freely within the ebonite stopper.

Its rods shall be straight and the wheels free from distortion and wear.

6.1.4 Precision viscometer tubes, manufactured from special glass, with the following dimensions:

— inner diameter: 21,00 mm ± 0,02 mm;

— outer diameter: 23,80 mm ± 0,25 mm;

— inner height: 220,0 mm ± 0,3 mm.

6.1.5 Rubber stoppers, to fit the viscometer tubes.

6.2 Automatic dispenser or **pipette**, ISO 8655-2[4], allowing a volume of 25,0 ml ± 0,2 ml to be delivered.

1) The “Falling Number” apparatus having a specifically designed viscometer stirrer produced by Perten Instruments is an example of a suitable device available commercially. This device forms the subject of a patent. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

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6.3 Analytical balance, capable of being read to the nearest 0,01 g.

6.4 Laboratory mill[2], hammer type, and fitted with a 0,8 mm screen allowing the production of a wholemeal product meeting the particle size specification shown in 8.1.3.

Check the performance of the mill periodically using a well-mixed sample of ground grain (as produced in 8.1.2).

The mill may be equipped with an automatic feeding device, particularly for the grinding of high moisture content grain.

6.5 Laboratory sieve, of nominal size of openings 800 µm, ISO 565 [1] and ISO 3310 (all parts)[2].

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 13690[5].

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Storage time and storage conditions of the sample in the laboratory may have a significant effect on FNs.

8 Preparation of test sample

8.1 Whole grain

8.1.1 Elimination of impurities

If necessary, clean the sample to eliminate impurities (e.g. stones, dust, husks and other cereal grains). Take a representative 300 g test sample from the laboratory sample.

A smaller test sample of about 200 g, although it provides less reproducible results, may be used for routine inspections. If the sample is less than 200 g, the results risk being marred by errors.

8.1.2 Grinding of grain samples

Feed the laboratory mill (6.4) carefully with grain to avoid heating and overloading. The feed to the mill may be controlled automatically with an automatic feeding device. Grinding should be continued for 30 s to 40 s after the last of the sample has entered the mill. Discard the bran particles remaining inside the mill, provided these do not represent more than 1 % of the quantity of grain sampled for grinding. Thoroughly mix all of the milled product before use.

It is recommended (especially in the case of successive grindings) to allow the meal to cool for 1 h before proceeding with the test.

8.1.3 Ground sample specification

WARNING — FNs can be affected by the particle size of the ground grain.

2) LM 3100 and LM 120 mills are examples of suitable devices 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.

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The milled product shall comply with the specification given in Table 1.

Table 1 — Ground sample specification

Nominal size of openings of sieve µm	Amount of ground sample passing through sieve %
710	100
500	95 to 100
200	80 or less

Check the particle size distribution of the ground material regularly using a well-mixed sample of ground grain (8.1.2).

To do this, select the appropriate sieves, as specified in Table 1, and arrange in order of decreasing nominal size of openings with a suitable sieving aid in each sieve and a receiver pan on the bottom. Weigh out a representative sample of 50,0 g and place on the top sieve. Sieve in a horizontal plane, manually for at least 5 min until nothing passes through the 710 µm sieve, or mechanically for a period of 10 min. Weigh the material retained on each sieve and that contained in the receiver pan. Calculate the percentage of ground grain which passes through each sieve.

8.2 Flour and semolina samples

Flour samples shall not contain lumps. If necessary, sift the flour using the laboratory sieve (6.5) in order to remove lumps or foreign bodies.

For coarse commercial wholemeal flours or semolina, grind a sample using the laboratory mill (6.4) in order to produce a test sample which conforms to the particle size specification shown in Table 1. Mix the ground sample thoroughly before use.

9 Procedure

9.1 Determination of the moisture content

Determine the FN on flour or ground material with a moisture content of 15 % mass fraction.

Determine the moisture content of the prepared test material (8.1 and 8.2) using the method specified in ISO 712.

Alternatively, a rapid instrumental procedure may be used (e.g. near infrared reflectance) provided this has been calibrated using ISO 712.

9.2 Test portion

Carry out the determination on two test portions simultaneously or rapidly one after the other.

Refer to Table 2, column (2) which shows the required mass of sample to be taken, at different moisture contents, in order to ensure that a constant ratio of dry matter is used for the FN determination.

If greater differentiation of the FNs is required for samples with very high α-amylase activity, as is normally the case for rye, refer to column (3).

Weigh the test portion to the nearest 0,05 g.

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Table 2 — Test portion as a function of moisture content of the sample

Moisture content %	Test portion, g for a nominal mass of 7 g at 15 % moisture content	Test portion, g for a nominal mass of 9 g at 15 % moisture content	Moisture content %	Test portion, g for a nominal mass of 7 g at 15 % moisture content	Test portion, g for a nominal mass of 9 g at 15 % moisture content
(1)	(2)	(3)	(1)	(2)	(3)
9,0	6,40	8,20	13,6	6,85	8,80
9,2	6,45	8,25	13,8	6,90	8,85
9,4	6,45	8,25	14,0	6,90	8,85
9,6	6,45	8,30	14,2	6,90	8,90
9,8	6,50	8,30	14,4	6,95	8,90
10,0	6,50	8,35	14,6	6,95	8,95
10,2	6,55	8,35	14,8	7,00	8,95
10,4	6,55	8,40	15,0	7,00	9,00
10,6	6,55	8,40	15,2	7,00	9,05
10,8	6,60	8,45	15,4	7,05	9,05
11,0	6,60	8,45	15,6	7,05	9,10
11,2	6,60	8,50	15,8	7,10	9,10
11,4	6,65	8,50	16,0	7,10	9,15
11,6	6,65	8,55	16,2	7,15	9,20
11,8	6,70	8,55	16,4	7,15	9,20
12,0	6,70	8,60	16,6	7,15	9,25
12,2	6,70	8,60	16,8	7,20	9,25
12,4	6,75	8,65	17,0	7,20	9,30
12,6	6,75	8,65	17,2	7,25	9,35
12,8	6,80	8,70	17,4	7,25	9,35
13,0	6,80	8,70	17,6	7,30	9,40
13,2	6,80	8,75	17,8	7,30	9,40
13,4	6,85	8,80	18,0	7,30	9,45

9.3 Determination of falling number

9.3.1 Fill the water bath (6.1.1) with water to the level dictated by the overflow. Turn on the cooling system and ensure that cold water is flowing through the cooling lid. Switch on the FN apparatus and bring the water to the boil. The water bath shall be boiling vigorously before any determinations are carried out and during the entire test period.

9.3.2 Transfer the weighed test portion (9.2) to a clean, dry viscometer tube (6.1.4). Add 25 ml $\pm$ 0,2 ml of water (5.1) at 22 °C $\pm$ 2 °C using the automatic dispenser or pipette (6.2).

9.3.3 Immediately stopper (6.1.5) the viscometer tube (6.1.4) and shake[3)] it vigorously up and down 20 to 30 times in order to obtain a uniform suspension. Ensure that dry flour or ground material is not trapped at the top of the tube against the stopper. If necessary, free trapped material by easing the stoppers slightly upwards and reshaking as necessary.

3) The “Shake-Matic” apparatus is an example of a suitable device 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.

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9.3.4 Remove the stopper (6.1.5), scrape any material remaining on the bottom of the stopper back into the tube (6.1.4) and, with the viscometer stirrer (6.1.3), scrape down any material adhering to the sides of the tube. Leave the stirrer in the tube.

For dual systems, operations 9.3.2 to 9.3.4 should be performed within 30 s of the addition of water and may be conducted on two tubes simultaneously.

9.3.5 Immediately place the viscometer tube (6.1.4), together with the stirrer (6.1.3), through the hole in the lid into the boiling water bath (6.1.1). Activate the stirrer head (single or double head) in accordance with the manufacturer's instructions. The equipment can then automatically carry out the operations to complete the test. The test is considered to be complete when the viscometer stirrer has reached the bottom of the gelatinized suspension. Record the time displayed on the timer (6.1.2). This constitutes the FN.

9.3.6 Swing the stirrer head across or press the “stop” button to withdraw the stirrer head. Remove the tube and its stirrer carefully as they are hot. Clean the tubes and stirrers thoroughly, ensuring that no material is left in the recess of the ebonite top which could interfere with the descending stirrer during the next test. Rinse the tubes and allow to drain. Ensure that the viscometer stirrer is dry before re-use.

9.4 Calculation

9.4.1 Falling Number

The FN is affected by the boiling temperature of the water, which is linked to the atmospheric pressure and the altitude of the laboratory. No adjustment of the boiling temperature of the water bath should be made as this leads to errors in the results.

For laboratories located below an altitude of 600 m, uncorrected FNs should be used for ground grain samples, and below an altitude of 750 m uncorrected values should be used for flour and semolina.

For those laboratories located above these altitudes, apply Equation (A.1) or (A.2), as appropriate.

Take as result the arithmetic mean of two determinations if the repeatability conditions given in Table 3 and Table 4 are satisfied.

9.4.2 Liquefaction number

The relationship between FNs and α-amylase activity is not linear and therefore FNs cannot be used to calculate the composition of mixtures of grain, flour or semolina. The relationship may be converted from non-linear to linear, which makes it possible to calculate the theoretical FN of a blend of wheat, flour or semolina arithmetically or graphically. Transform an FN into an LN, n_L, using the empirical Equation (1):

$$n_L = \frac{6\,000}{t - 50} \tag{1}$$

where

t is the FN;

6 000 is a constant;

50 is a constant, corresponding approximately to the time, expressed in seconds, required for the starch to gelatinize sufficiently to be susceptible to attack by enzymes.

LNs are proportional to α-amylase activity over the normal range found in commercial flour.

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10 Precision

10.1 Interlaboratory tests

Results of interlaboratory tests on the precision of the method are given in Annex B. The values derived from these interlaboratory tests may not be applicable to concentration ranges or 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, shall in not more than 5 % of cases be greater than the values given in Table 3 and Table 4.

If the results of two tests are outside these limits, carry out two determinations again.

10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, shall in not more than 5 % of cases be greater than the values given in Table 3 and Table 4.

Table 3 — Repeatability and reproducibility limits for falling numbers for flour (deduced from Table B.1)

Values in seconds

Falling number	Repeatability limit r	Reproducibility limit R
60 to 199	5	10
200 to 229	9	24
230 to 259	12	27
260 to 289	15	30
290 to 319	19	33
320 to 349	22	36
350 to 379	25	39
380 to 409	28	42
410 to 439	31	45
440 to 469	35	48
470 to 499	38	51
$\geqslant$ 500	40	60

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Table 4 — Repeatability and reproducibility limits for falling numbers for wheat (deduced from Table B.2)

Values in seconds

Falling number	Repeatability limit r	Reproducibility limit R
60 to 79	10	10
80 to 109	13	21
110 to 139	15	30
140 to 169	17	38
170 to 199	19	46
200 to 229	21	54
230 to 259	23	62
260 to 289	25	70
290 to 319	27	78
320 to 349	30	86
350 to 379	32	94
$\geqslant$ 380	40	100

11 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, including a reference to this International Standard (ISO 3093:2009), and in particular the nominal mass of the test portion taken;

d) 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);

e) the test result(s) obtained, and whether any correction has been made for altitude;

f) if the repeatability has been checked, the final result obtained.

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Annex A
(normative)

Equations for altitude correction of falling numbers

A.1 Ground grain samples

For laboratories located at an altitude above 600 m, where the boiling temperature of the water bath is lower than 98 °C, calculate the FN at sea level, t_0, using Equation (A.1):

$$t_0 = 10^{X_1} \tag{A.1}$$

where

X_1 = (1,0 × lg t_H) – (4,972 35 × 10^{-5} × H) + (2,449 96 × 10^{-9} × H^2) + (1,753 14 × 10^{-5} × lg t_H × H) – (9,938 495 × 10^{-10} × lg t_H × H^2)

in which

t_H is the uncorrected value measured at altitude, H;

H is the altitude, in metres, above sea level of the laboratory.

A.2 Flour and semolina

For laboratories located at an altitude above 750 m, where the boiling temperature of the water bath is lower than 98 °C, calculate the FN at sea level, t_0, using Equation (A.2):

$$t_0 = 10^{X_2} \tag{A.2}$$

where

X_2 = – 849,41 + (3,956 0 × 10^{-7} × H^2) + (454,19 × lg t_H) – (1,978 9 × 10^{-7} × lg t_H × H^2)

in which

t_H is the uncorrected value measured at altitude, H;

H is the altitude, in metres, above sea level of the laboratory.

The calculation of FN from Equations (A.1) and (A.2) may be replaced by reading from a conversion table, drawn up to correct for a specific altitude, in order that corrected FNs may be obtained for each measured value.

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Annex B
(informative)

Results of interlaboratory tests

B.1 Wheat flours

A test conducted by the International Association for Cereal Science and Technology (ICC) between 11 laboratories (of which results from 10 were retained) on 10 samples of flours gave the statistical results (assessed according to ICC 107/1[6]) shown in Table B.1.

Table B.1 — Data for falling number for wheat flour

Values in seconds

Parameter	Sample									
	1	2	3	4	5	6	7	8	9	10
Mean value	210,6	218,6	244,5	263,9	296,5	333,5	373,9	408,3	457,2	485,9
Repeatability standard deviation, s_r	4,51	4,22	5,03	4,73	5,28	6,74	8,31	10,96	10,88	16,70
Relative standard deviation of repeatability, %	2,14	1,93	2,06	1,79	1,78	2,02	2,22	2,68	2,38	3,44
Repeatability limit, r (2,8 s_r)	12,63	11,82	14,08	13,25	14,79	18,88	23,27	30,70	30,48	46,76
Reproducibility standard deviation, s_R	5,66	9,12	10,21	8,20	14,98	16,00	13,23	17,82	13,81	18,89
Relative standard deviation of reproducibility, %	2,69	4,17	4,18	3,11	5,05	4,80	3,54	4,37	3,02	3,89
Reproducibility limit, R (2,8 s_R)	15,84	25,53	28,59	22,97	41,94	44,82	37,04	49,91	38,67	52,90

The relative standard deviation of repeatability is less than or equal to 3,44 %.

The relative standard deviation of reproducibility is less than or equal to 5,05 %.

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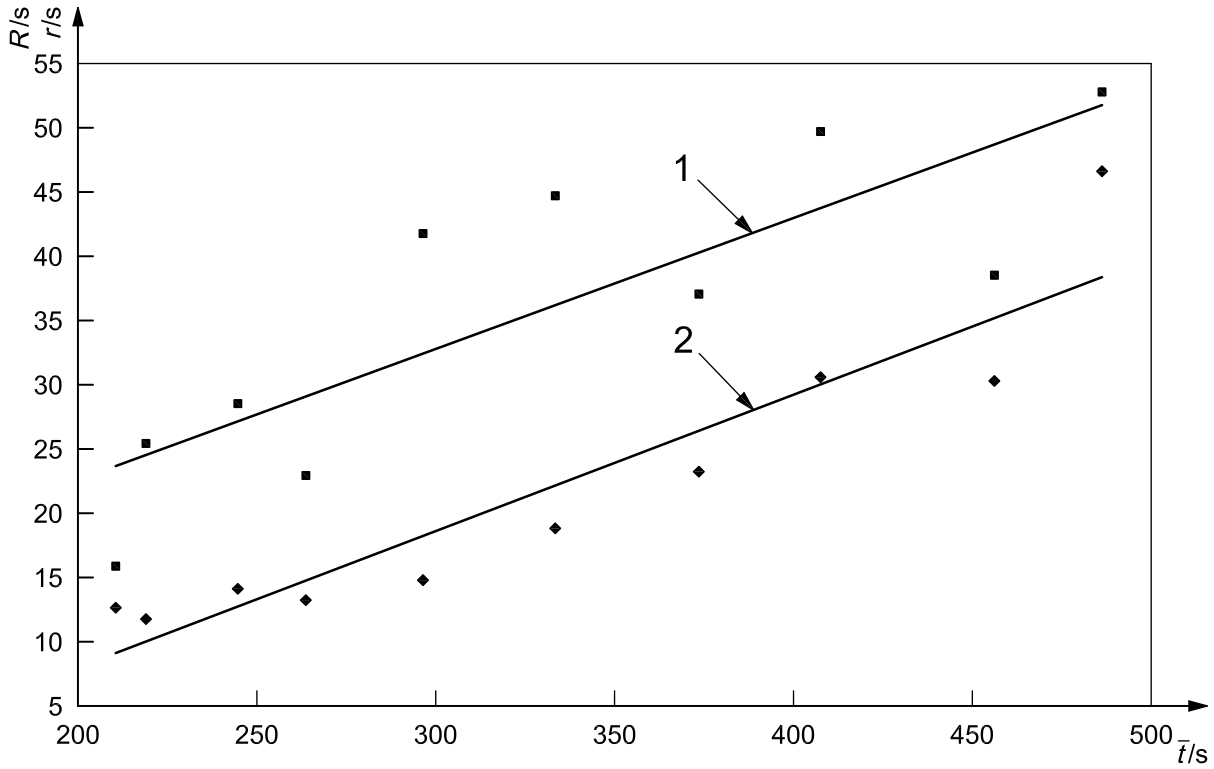

Key

$\bar{t}$ mean FN

R reproducibility limit

r repeatability limit

1 reproducibility equation: $R = 0{,}102\,5\ \bar{t} + 2{,}103$

$r^2_{\bar{t}R} = 0{,}689$

where $r_{\bar{t}R}$ is the correlation coefficient for the reproducibility equation

2 repeatability equation: $r = 0{,}106\,9\ \bar{t} - 13{,}547$

$r^2_{\bar{t}r} = 0{,}872\,5$

where $r_{\bar{t}r}$ is the correlation coefficient for the repeatability equation

Figure B.1 — Repeatability and reproducibility curve for wheat flours

B.2 Wheats

A test conducted by the Bureau Interprofessionnel d'Études Analytiques (BIPEA) between 11 laboratories (of which results from 10 were retained) on three wheat samples gave the statistical results (assessed according to ISO 5725:1986 [3]) shown in Table B.2.

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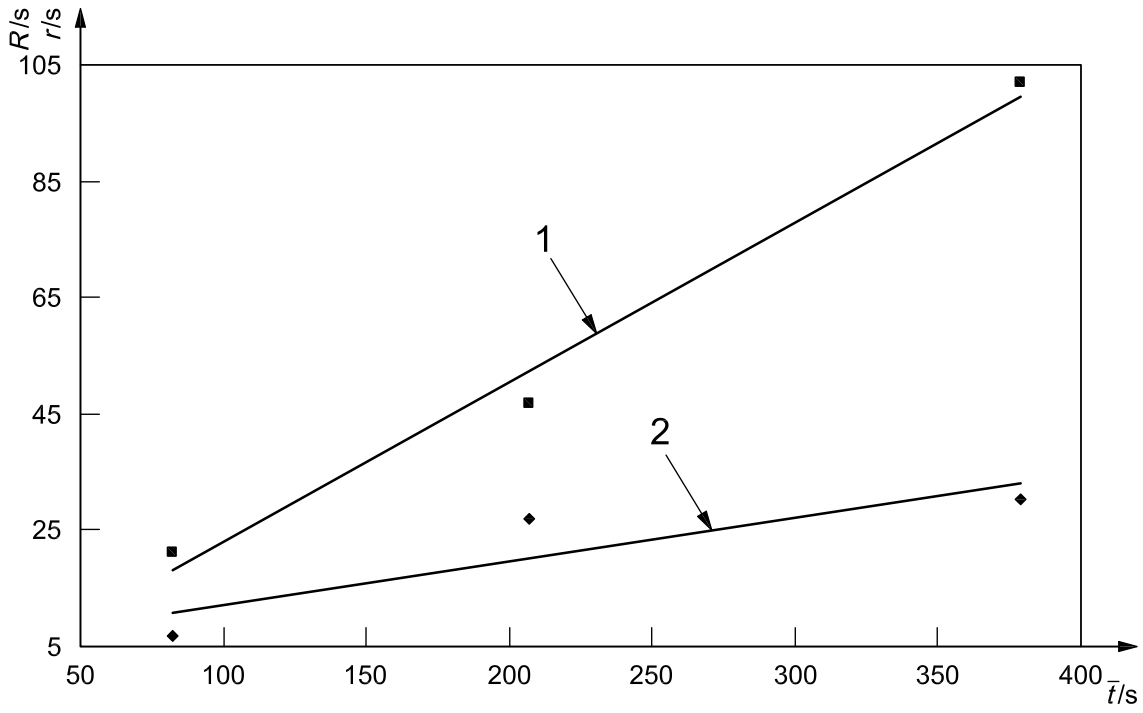
Table B.2 — Data for falling number for wheat

Values in seconds

Parameter	Sample		
	1	2	3
Mean value	82,1	207,1	379,1
Repeatability standard deviation, s_r	2,5	9,5	10,7
Relative standard deviation of repeatability, %	3	4,6	2,8
Repeatability limit, r (= 2,8 s_r)	6,9	26,9	30,3
Reproducibility standard deviation, s_R	7,5	16,5	36,0
Relative standard deviation of reproducibility, %	9,2	8,0	9,5
Reproducibility limit, R (= 2,8 s_R)	21,3	46,7	101,8

The relative standard deviation of repeatability is less than or equal to 4,6 %.

The relative standard deviation of reproducibility is less than or equal to 9,5 %.



Key

$\bar{t}$ mean FN
R reproducibility limit
r repeatability limit
1 reproducibility equation: $R = 0{,}274\ \bar{t} - 4{,}445$
$r^2_{\bar{t}R} = 0{,}986$
where $r_{\bar{t}R}$ is the correlation coefficient for the reproducibility equation
2 repeatability equation: $r = 0{,}075\ 2\ \bar{t} + 4{,}612$
$r^2_{\bar{t}r} = 0{,}786\ 9$
where $r_{\bar{t}r}$ is the correlation coefficient for the repeatability equation

Figure B.2 — Repeatability and reproducibility curve for wheat

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Bibliography

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

[2] ISO 3310 (all parts), *Test sieves — Technical requirements and testing*

[3] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests* [4)]

[4] ISO 8655-2, *Piston-operated volumetric apparatus — Part 2: Piston pipettes*

[5] ISO 13690, *Cereals, pulses and milled products — Sampling of static batches*

[6] ICC 107/1, *Determination of the "falling number" according to Hagberg-Perten as a measure of the degree of alpha-amylase activity in grain and flour*

4) Superseded by ISO 5725-1:1994 and ISO 5725-2:1994.

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