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Wheat and wheat flour — Gluten content —

Part 2: Determination of wet gluten by mechanical means

Blé et farines de blé — Teneur en gluten —

Partie 2: Détermination du gluten humide par des moyens mécaniques

Reference number
ISO 21415-2:2006(E)

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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.

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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 21415-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

This first edition of ISO 21415-2 cancels and replaces ISO 7495:1990, which has been technically revised. Together with ISO 21415-1:2006, it also cancels and replaces ISO 5531:1978.

ISO 21415 consists of the following parts, under the general title *Wheat and wheat flour — Gluten content*:

— *Part 1: Determination of wet gluten by a manual method*

— *Part 2: Determination of wet gluten by mechanical means*

— *Part 3: Determination of dry gluten from wet gluten by an oven drying method*

— *Part 4: Determination of dry gluten from wet gluten by a rapid drying method*

Introduction

The alternative techniques specified in this part of ISO 21415 and in ISO 21415-1 for the isolation of wet gluten (i.e. washing out by hand and mechanical washing out) do not usually give equivalent results. The reason is that, for complete development of gluten structure it is necessary to rest the dough. Therefore, the result obtained by hand washing is usually higher than that obtained by mechanical washing, mainly in the case of wheat which has high gluten content. Consequently, the test report should always indicate the technique used.

Wheat and wheat flour — Gluten content —

Part 2: Determination of wet gluten by mechanical means

1 Scope

This part of ISO 21415 specifies a method for the determination of the wet gluten content of wheat flour (*Triticum aestivum* L. and *Triticum durum* Desf.) by mechanical means. This method is directly applicable to flour. It is also applicable to semolina and wheat after grinding, if their particle size distribution meets the specification given in Table B 1.

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*

3 Terms and definitions

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

3.1
wet gluten
visco-elastic substance, composed principally of two protein fractions (gliadin and glutenin) in hydrated form, obtained as specified in this part of ISO 21415 or ISO 21415-1

3.2
ground wheat
product of small-scale milling of whole wheat which meets the particle size distribution shown in Table B.1.

3.3
semolina
coarsely milled wheat endosperm

3.4
flour
finely milled wheat endosperm with a particle size of less than 250 µm

4 Principle

Dough is prepared from a sample of flour or reground semolina, or ground wheat and a solution of sodium chloride, in the chamber of the equipment. The wet gluten is separated by washing the dough with sodium chloride solution, followed by removal of excess washing solution by centrifugation. The residue is weighed.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

5.1 Sodium chloride solution, 20 g/l.

Dissolve 200 g of sodium chloride (NaCl) in water then dilute it to 10 l. The temperature of the solutions when used should be 22 °C ± 2 °C.

It is advisable to prepare this solution freshly every day.

5.2 Potassium iodide/iodine solution (Lugol's solution).

Dissolve 2,54 g of potassium iodide (KI) in water. Add 1,27 g of iodine (I_2) to this solution and, after complete dissolution of the integral parts, dilute to 100 ml with water.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Automatic apparatus for gluten separation[1)], single or double, consisting of washing chamber(s), kneader(s) (see Figures A.1 and A.2) and a dispensing device under electronic control for gluten separation.

6.1.1 Washing chamber(s), equipped with interchangeable chromed sieve holder(s) and with 88 µm aperture polyester sieves or 80 µm aperture metal sieves, and 840 µm aperture polyamide sieves or 800 µm aperture metal sieves.

6.1.2 Kneader hook, at a distance from the chromed sieve holder of 0,7 mm ± 0,05 mm. This value should be checked using the stamped metal plates supplied.

6.1.3 Plastic container, of capacity 10 l, containing a reservoir of sodium chloride solution (5.1) connected to the equipment by plastic tubing.

6.1.4 Dispensing device, consisting of a peristaltic pump, to deliver the sodium chloride solution (5.1) for gluten washing at a constant flow rate of 50 ml/min to 56 ml/min.

For a detailed description of the equipment and for detailed operating instructions, users of this part of ISO 21415 should consult the manufacturer's manual for the equipment employed.

6.2 Adjustable dispenser, for the sodium chloride solution, capable of dispensing 3 ml to 10 ml with an accuracy of ± 0,1 ml.

6.3 Centrifuge, capable of maintaining a rotational frequency of 6 000 ± 5 per minute and of producing a radial acceleration of 2 000 g, equipped with perforated plates having apertures 500 µm in diameter.

6.4 Balance, capable of weighing to the nearest 0,01 g.

6.5 Spatula, made of stainless steel.

6.6 Beakers, of capacity 500 ml (to collect washings).

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1) Glutomatic equipment (types 2100 and 2200) produced by Perten Instruments AB (Sweden) is the most widely used mechanical means for this purpose. This information is given for the convenience of users of this part of ISO 21415 and does not constitute an endorsement by ISO of this equipment. Other equipment may also be used if it gives similar results to Glutomatic equipment or to the method specified in ISO 21415-1.

6.7 Metal tweezers.

6.8 Small-scale mill, capable of producing ground product with a particle size that meets the requirements given in Table B.1.

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 a part of the method specified in this part of ISO 21415. A recommended sampling method is given in ISO 6644 or ISO 13690.

8 Preparation of test sample

Homogenize the samples and determine their moisture content in accordance with ISO 712. Prior to the gluten content measurement, grind the wheat grain and semolina using a small-scale mill (6.8) as specified in Annex B. To avoid significant changes in the moisture content of the samples, take special care during grinding and storage.

9 Procedure

9.1 General

The operations of preparation and washing of the dough are carried out as a continuous process by the automatic apparatus (6.1). Work in accordance with the manufacturer's operating instructions for the apparatus in use.

9.2 Test portion

Weigh 10 g of the test sample to the nearest 0,01 g and transfer it quantitatively to the washing chamber of the apparatus (6.1.1). Make sure that the washing chamber is equipped with the appropriate sieve, which has been previously cleaned and moistened.

Fine (88 µm aperture) polyester or (80 µm aperture) metal sieve(s) is/are used for testing flour and reground semolina samples. When testing ground wheat, chromed sieve holder(s) with an indented ring around the base is/are also required with 840 µm aperture polyamide or 800 µm metal sieve(s). The aperture size of the sieve(s) used should be indicated in the test report.

Shake the chamber gently to spread the flour evenly.

9.3 Dough preparation

As a suggested starting point, add 4,8 ml of the sodium chloride solution (5.1) to the test sample using the adjustable dispenser (6.2). Direct the stream of salt solution against the side wall of the chamber so that it does not go through the sieve. Shake the washing chamber gently so that the salt solution is spread evenly over the flour.

It may be necessary to adjust the amount of salt solution used to accommodate high and low gluten content or very weak gluten samples. Where difficulties in mixing cohesive dough are experienced (the chamber is flooded during the washing period), the amount of added salt solution should be reduced (minimum of 4,2 ml). Where a very strong, tough gluten is formed during mixing, the amount of solution may be increased to 5,2 ml.

The preparation time is preset by the manufacturer at 20 s, but may be adjusted by the user if necessary. In the latter case, consult the manufacturer for information on the adjustment of the timer.

9.4 Washing the dough

9.4.1 General

During the washing process, observe the clarity of the effluent leaving the washing chamber. The dough is considered to have been washed sufficiently when the effluent becomes clear. Check by using Lugol's solution (5.2) that the effluent is free from starch.

9.4.2 For flour or reground semolina

The washing time is preset by the manufacturer at 5 min. A volume of between 250 ml and 280 ml of sodium chloride solution is usually required during the washing operation. This solution is delivered automatically by the apparatus at a preset constant flow rate of between 50 ml/min and 56 ml/min (depending on the apparatus).

9.4.3 For ground wheat

After 2 min washing, stop the apparatus, remove the washing chamber with the partially washed out gluten and transfer the total content, including bran particles, to another washing chamber containing a coarse (840 µm) sieve. This may be done by placing the washing chamber under gently running cold water (open end to open end and with the fine sieve uppermost).

Place the washing chamber with the coarse sieve, containing the transferred gluten ball, in the working position and continue the washing while the remainder of the washing sequence is completed.

9.4.4 Special case

If the automatic washing process does not achieve sufficient washing of the dough, carry out one of the following operations:

a) either during the washing process, manually add excess sodium chloride solution to the washing chamber, or

b) adjust the apparatus to repeat the washing process.

9.5 Removal of excess water and weighing the wet gluten

When the washing process has been completed, remove the wet gluten from the washing chamber using the metal tweezers (6.7). Ensure that no gluten remains in the washing chamber.

Divide the gluten into two approximately equal portions and place the balls of gluten on the perforated plates of the centrifuge (6.3), pressing them down gently.

Operate the centrifuge to remove the excess solution from the gluten (the preset time is 60 s). Remove the gluten piece(s) using the metal tweezers (6.7) and immediately weigh the total (m_1) to the nearest 0,01 g.

NOTE Dividing the gluten is not necessary if a counterbalance is used in the centrifuge.

If using a double instrument, two gluten pieces are produced. These should be treated separately in the subsequent stages.

9.6 Number of determinations

Carry out two determinations on the same sample.

10 Calculation and expression of results

The wet gluten content (G_{wet}), expressed as a mass fraction of the test sample in percent, is calculated by the following equation:

$$G_{wet} = m_1 \times 10\ \%$$

where m_1 is the mass of wet gluten (see 9.5), in grams.

Take as a result the arithmetic mean of two determinations if the repeatability conditions (see 11.2) are fulfilled. Express the result to one decimal place.

11 Precision

11.1 Interlaboratory tests

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

11.2 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 than 5 % of cases be greater than the values given below:

— for wheat as grain: r = 1,9 g/100 g;

— for wheat flour: r = 1,0 g/100 g;

— for durum wheat as grain: r = 1,6 g/100 g;

— for durum wheat semolina: r = 1,6 g/100 g.

11.3 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, will in not more than 5 % of cases be greater than the values given below:

— for wheat as grain: R = 4,0 g/100 g;

— for wheat flour: R = 2,4 g/100 g;

— for durum wheat as grain: R = 5,8 g/100 g;

— for durum wheat semolina: R = 10,1 g/100 g.

12 Test report

The test report shall specify:

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

b) the sampling method used, if known;

c) the test method used with reference to this part of ISO 21415, including details of the milling procedure, and sieve meshes used during gluten isolation;

d) all operating details not specified in this part of ISO 21415, or regarded as optional, together with details of any incidents that occurred when performing the method, which may have influenced the test result;

e) the result obtained;

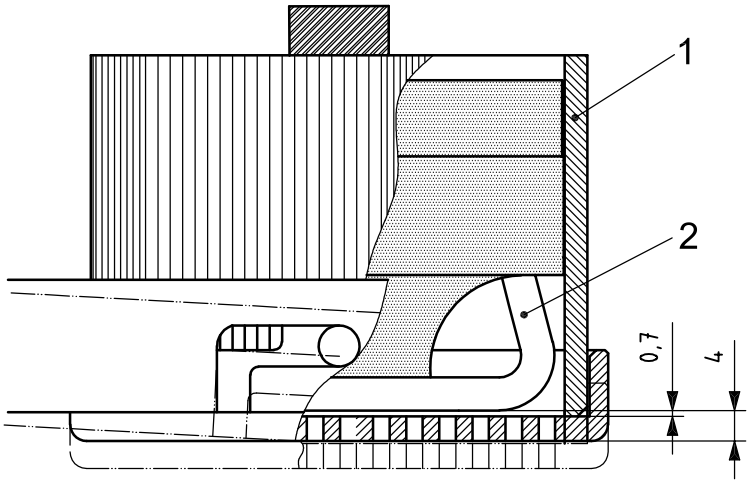
f) if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Washing chamber and kneader of Glutomatic equipment, and the centrifuge

The equipment shown in Figures A.1 to A.2 is as given in Reference [6].

Dimensions in millimetres



Key

1 mixing/washing chamber
2 kneader

Figure A.1 — Apparatus for gluten separation

Dimensions in millimetres

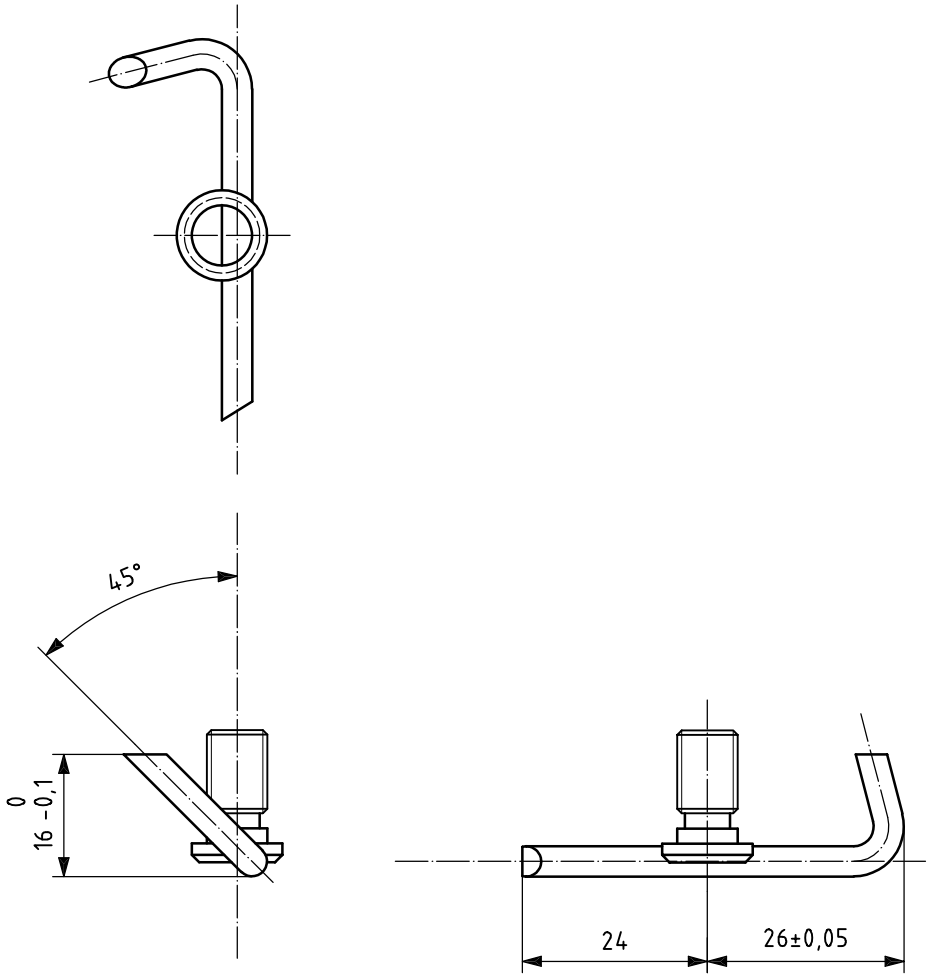


Figure A.2 — Kneader

Annex B
(normative)

Preparation of ground wheat

As mentioned in the Scope, the method is also applicable to wheat and semolina after grinding in a small-scale mill (6.8).

Gluten formation and washing are affected by the particle size of the ground samples. The mill used shall be capable of grinding to the specification shown in Table B.1.

Table B.1 — Aperture of sieves and the required particle size distribution of samples

Aperture of sieve µm	Sample passing through sieve %
710	100
500	95 to 100
210 to 200	80 or less

It should be emphasized that Table B.1 is used for regular adjustment of the mill. The whole grinding is subject to the type of determination. The particle size distribution should be checked regularly, using a well-mixed ground sample and a suitable laboratory sifter.

The grinding method to prepare a ground sample from wheat or semolina influences the results of the gluten determination. Different mills produce different ground sample fractions of different composition, which will cause variation in dough formation and the washed out gluten. To obtain comparable results, the same method of sample preparation shall be used. The grinding procedure to produce ground sample shall be reported together with the percentage of gluten.

A representative sample of wheat or semolina shall be ground to meet the particle size specification shown in Table B.1. The mill shall be fed carefully with wheat or semolina to avoid heating and overloading. Grinding should be continued for 30 s to 40 s after the last of the sample has entered the mill. Small quantities (up to 1 %) of the wheat or semolina should be taken for grinding.

Annex C
(informative)

Results of an interlaboratory test

An interlaboratory test involving 21 laboratories in 7 countries was organized by the CONCORDIA Warehouse Ltd., Grain Control Laboratory, Budapest (Hungary) in 2004. It was carried out on the following six samples:

— Sample A: wheat (*Triticum aestivum* L.) as grain;

— Sample B: wheat (*Triticum aestivum* L.) as grain;

— Sample C wheat (*Triticum durum* Desf.) as grain;

— Sample D durum wheat semolina;

— Sample E wheat flour;

— Sample F wheat flour.

The results obtained were subjected to statistical analysis in accordance with ISO 5725-1 and ISO 5725-2 to give the precision data shown in Table C.1.

Table C.1 — Precision data for wet gluten determined by ISO 21415-2

	Samples					
	A	**B**	**C**	**D**	**E**	**F**
Number of laboratories after eliminating outliers	19	19	17	18	18	18
Mean value, g/100 g	26,06	34,61	30,46	36,89	27,32	35,17
Repeatability standard deviation, s_r, g/100 g	0,66	0,62	0,57	0,57	0,30	0,35
Coefficient of variation of repeatability, %	2,53	1,79	1,86	1,57	1,09	0,99
Repeatability limit r (= 2,8 s_r), g/100 g	1,85	1,74	1,59	1,59	0,83	0,97
Reproducibility standard deviation, s_R, g/100 g	1,43	1,45	2,06	3,61	0,75	0,85
Coefficient of variation of reproducibility, %	5,49	4,18	6,75	9,79	2,73	2,42
Reproducibility limit R (= 2,8 s_R), g/100 g	4,00	4,05	5,76	10,12	2,09	2,38

Bibliography

[1] ISO 5725-1*, Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[2] ISO 5725-2*, Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[3] ISO 6644, *Flowing cereals and milled cereal products — Automatic sampling by mechanical means*

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

[5] ISO 21415-1, *Wheat and wheat flour — Gluten content — Part 1: Determination of wet gluten by a manual method*

[6] ICC Standard No.137/1:1994, *Mechanical Determination of the Wet Gluten Content of Wheat Flour (Glutomatic)*

[7] ICC Standard No. 155:1994, *Determination of Wet Gluten Quantity and Quality (Gluten Index ac. to Perten) of Whole Wheat Meal and Wheat Flour (*Triticum aestivum*)*

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