
**Wheat and wheat flour — Gluten
content —**

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

Blé et farines de blé — Teneur en gluten —

Partie 1: Détermination du gluten humide par une méthode manuelle

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Contents

Page

Foreword.....	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions.....	1
4 Principle.....	1
5 Reagents	2
6 Apparatus	2
7 Sampling.....	3
8 Preparation of test sample.....	3
9 Procedure	3
9.1 General.....	3
9.2 Test portion	3
9.3 Dough preparation and leaving it to rest.....	3
9.4 Washing out	3
9.5 Removal of excess washing solution.....	4
9.6 Determination of the mass of the wet gluten.....	4
9.7 Number of determinations	4
10 Calculation and expression of results.....	4
11 Precision.....	5
11.1 Interlaboratory tests	5
11.2 Repeatability.....	5
11.3 Reproducibility	5
12 Test report	5
Annex A (normative) Gluten press	6
Annex B (normative) Preparation of ground wheat.....	7
Annex C (informative) Results of an interlaboratory test.....	8
Bibliography	9

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 21415-1 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

This first edition of ISO 21415-1, together with ISO 21415-2 (to be published), cancels and replaces ISO 5531:1978, which has been technically revised.

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 21415-2 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 the 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.

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Wheat and wheat flour — Gluten content —

Part 1: Determination of wet gluten by a manual method

1 Scope

This part of ISO 21415 specifies a manual washing out method for the determination of the wet gluten content of wheat flour (*Triticum aestivum* L. and *Triticum durum* Desf.). 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 a hydrated form, obtained as specified in this part of ISO 21415 or ISO 21415-2

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. The dough is left to rest for the gluten structure to develop. The wet gluten is separated by hand

washing the dough with sodium chloride solution, followed by removal of excess washing solution. 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.

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 (I₂) 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 **Porcelain mortar**, glazed inside, or **enamelled metal vessel** of diameter 10 cm to 15 cm.

6.2 **Burette**, of capacity 25 ml, graduated in 0,1 ml.

6.3 **Beaker**, of capacity 250 ml.

6.4 **Spatula**, plastic or stainless steel, 18 cm to 20 cm in length.

6.5 **Glass plate**, about 40 cm × 40 cm.

6.6 **Gloves**, of thin rubber and having a smooth surface.

6.7 **Wooden frame**, about 30 cm × 40 cm, covered with No. 56 grit gauze (308 µm).

6.8 **Container**, with adjustable outflow, for the sodium chloride solution (5.1).

6.9 **Filter paper**¹⁾, of mass approximately 120 g/m².

6.10 **Stop-watch**.

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

6.12 **Gluten press**, see Annex A.

6.13 **Watch-glass**, of diameter 8 cm.

6.14 **Small-scale mill**, capable of milling to a particle size distribution that meets the requirements given in Table B.1.

1) For example, the Macherey Nagel No. 651 filter paper is suitable 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 product.

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 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 wheat grain and semolina using a small-scale mill (6.14) as specified in Annex B. To avoid changes in the moisture content of the samples, take special care during grinding and storage.

9 Procedure

9.1 General

Use sodium chloride solution (5.1) for the preparation and washing of the dough.

The test sample and the sodium chloride solution should be kept at least for one night in the laboratory where the procedure will be carried out. If the ambient temperature is lower than 20 °C or higher than 25 °C, the temperature of the test sample and the sodium chloride solution should be adjusted to between 20 °C and 25 °C.

9.2 Test portion

Weigh about 24 g of the test sample to the nearest 0,01 g (m_1) and transfer it quantitatively to the mortar or vessel (6.1).

9.3 Dough preparation and leaving it to rest

9.3.1 Add drop-by-drop 12 ml of the sodium chloride solution (5.1) from the burette (6.2), while continuously stirring the flour with the spatula (6.4).

9.3.2 After adding the sodium chloride solution, compress the mixture with the spatula and form a dough ball, taking care to avoid loss of flour. Dough residues adhering to the wall of the vessel or to the spatula shall be collected with the dough ball.

9.3.3 Preparation of the dough should not take longer than 3 min.

9.3.4 Place the dough ball on the glass plate (6.5). Cover the inside of a 250 ml beaker (6.3) with wet filter paper (6.9) and then use it to cover the dough ball. Leave the dough to rest for 30 min.

9.4 Washing out

9.4.1 The operations described in 9.4.2 and 9.4.3 shall be carried out over the wooden frame covered with gauze (6.7) to avoid the possible loss of dough. During the operations, the hands shall be covered with rubber gloves (6.6) in order to protect the dough from warmth and perspiration from the hands.

9.4.2 After the resting period, weigh about 30 g from the dough ball (9.3) to the nearest 0,01 g (m_2). Take it in the palm of one hand and allow the sodium chloride solution (5.1) to drip onto it from the container (6.8) at a flowing rate of 750 ml per 8 min. During this time, successively roll out and mould the dough ball with the thumb of the other hand.

9.4.3 The washing is considered to be complete when the sodium chloride solution pressed out from the gluten ball obtained as in 9.4.2 is practically free of starch. For the detection of starch, press out some drops of the washing solution from the gluten ball into a watch-glass (6.13) and add a few drops of potassium iodide/iodine solution (5.2) to it. If the colour of the solution does not change, the washing out procedure is completed. If the colour of the solution turns blue, this indicates that starch is still present and the washing out procedure should be continued until it cannot be detected.

9.4.4 The washing out time depends on the gluten content, but in general is about 8 min.

9.5 Removal of excess washing solution

9.5.1 Eliminate most of the washing solution adhering to the gluten ball by holding it between the fingers of one hand and compressing it briefly three times.

9.5.2 Divide the gluten ball into two approximately equal parts, form them into a laminated shape and place them in the gluten press (6.12). Close the gluten press and re-open it after 5 s. Transfer the gluten leaves, without deforming them, to other dry places in the gluten press and close it again for 5 s. Repeat this operation 15 times. Dry the glass plates of the gluten press after each operation.

9.6 Determination of the mass of the wet gluten

Weigh together the two gluten leaves formed in 9.5.2, to the nearest 0,01 g (m_3).

9.7 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 equal to

$$G_{\text{wet}} = \frac{m_3(m_1 + 12)}{m_2 \times m_1} \times 100 \%$$

where

m_1 is the mass of test portion of the sample (9.2), in grams;

m_2 is the mass portion of dough ball (9.3) used for the washing out procedure (9.4), in grams;

m_3 is the mass of wet gluten (9.6), in grams;

12 is the volume of sodium chloride solution (5.1) used for preparing the dough, in millilitres.

Express the results to one decimal place.

NOTE 1 Deviation of the specific gravity of sodium chloride solution (5.1) from 1,00 is negligible in comparison with the standard deviation of the method itself.

NOTE 2 Generally the result of the determination is not expressed as the dry matter content of the test sample (flour or semolina or wheat). It is advisable, therefore, to give the moisture content of the test sample in the test report.

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: $r = 1,9 \text{ g/100 g}$;
- for wheat flour: $r = 1,5 \text{ g/100 g}$;
- for durum wheat as grain: $r = 0,9 \text{ g/100 g}$;
- for durum wheat semolina: $r = 2,1 \text{ g/100 g}$.

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: $R = 9,5 \text{ g/100 g}$;
- for wheat flour: $R = 7,7 \text{ g/100 g}$;
- for durum wheat as grain: $R = 14,0 \text{ g/100 g}$;
- for durum wheat semolina: $R = 11,7 \text{ g/100 g}$.

12 Test report

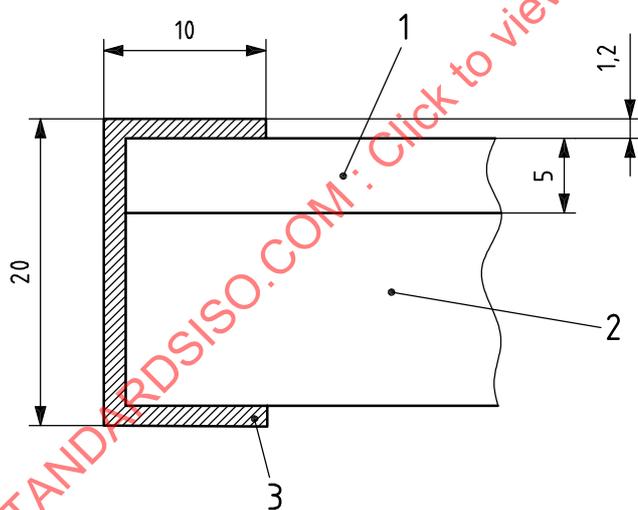
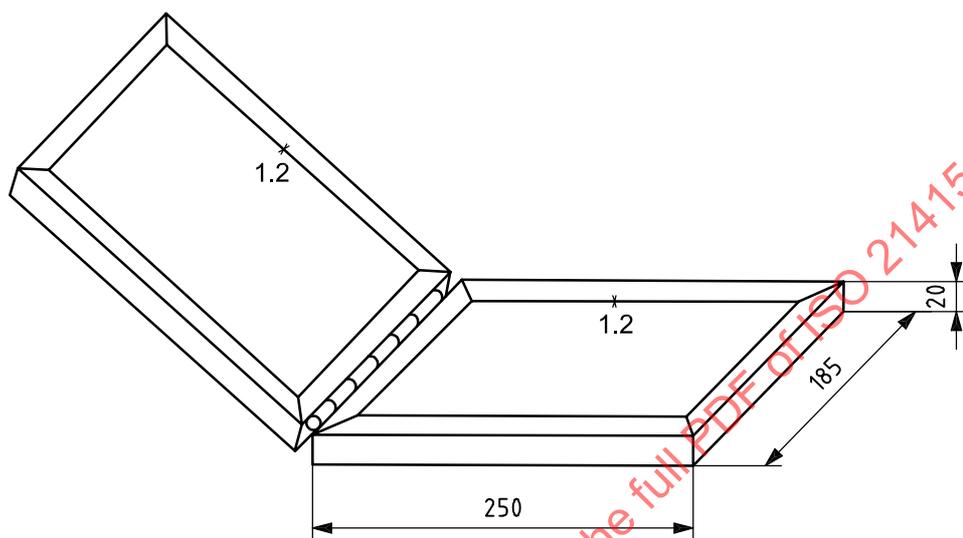
The test report shall specify:

- 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 grinding procedure;
- 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(s);
- e) the test result(s) obtained;
- f) if the repeatability has been checked, the final quoted result obtained.

Annex A
(normative)

Gluten press

Dimensions in millimetres



Key

- 1 glass plate with roughened surface
- 2 support
- 3 metal rim

NOTE Taken from Reference [4].