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Wheat and wheat flour — Gluten content —

Part 2: Determination of wet gluten and gluten index by mechanical means

Blé et farines de blé — Teneur en gluten —

Partie 2: Détermination du gluten humide et du gluten index par des moyens mécaniques

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ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

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Foreword

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The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

This second edition of ISO 21415-2 cancels and replaces the first edition (ISO 21415-2:2006) 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 and gluten index by mechanical means*

— *Part 3: Determination of dry gluten from wet gluten by using 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 isolation of wet gluten (i.e. manual extraction and mechanical extraction) do not generally yield equivalent results. The reason for this is that for full development of the gluten structure the dough needs to be allowed to rest. Hence, the result obtained by manual extraction is usually greater than that obtained by mechanical extraction, especially in the case of wheat with high gluten content. Therefore, the test report should always state the technique used.

Wheat and wheat flour — Gluten content —

Part 2: Determination of wet gluten and gluten index by mechanical means

1 Scope

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

2 Terms and definitions

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

2.1
wet gluten
viscoelastic substance consisting mainly of two protein fractions (gliadin and glutenin) in hydrated form, obtained in the way indicated in this part of ISO 21415 or in ISO 21415-1

2.2
gluten index
proportion of wet gluten remaining on the sieve after centrifugation

Note 1 to entry: The higher the index, the stronger the gluten is.

2.3
ground wheat
result of experimental grinding of whole wheat with the granulometry cited in Table B.1

2.4
flour
finely ground wheat endosperm with a granulometry of less than 250 µm

3 Principle

Preparation of a paste from a sample of flour or of ground wheat and a sodium chloride solution in the equipment's chamber; separation of the wet gluten by washing this paste with a sodium chloride solution, followed by removal of excess washing solution by centrifugation and weighing the residue. The gluten index is obtained after centrifuging to force the wet gluten through a special sieve. The percentage of wet gluten remaining on the sieve after centrifuging is defined as the gluten index.

4 Reagents

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

4.1 Sodium chloride solution, 20 g/l.

Dissolve 200 g of sodium chloride (NaCl) in water, then dilute to 10 l. When using this, it is recommended that the temperature of the solution be 22°C ± 2 °C.

It is advisable to prepare this solution specially every day.

4.2 Solution of iodine/potassium iodide (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, when the reagents have completely dissolved, dilute to 100 ml with water.

5 Apparatus

Usual laboratory apparatus and, in particular, the following items.

5.1 Automatic gluten separation unit [1], (single or double) consisting of a washing/mixing chamber, mill(s) (see Figures A.1 and A.2) and an electronically controlled distribution device for extracting the gluten.

5.1.1 Mixing/washing chamber(s), fitted with replaceable chrome-plated sieve support(s) with polyester sieves with an 88 µm mesh gap and polyamide sieves with an 840 µm mesh gap.

The distance between the milling hook and the chrome-plated sieve support shall be 0,7 mm ± 0,05 mm. This value should be checked with the calibrated thickness shims supplied.

5.1.2 10 l plastic drum, to contain the sodium chloride solution (4.1) connected to the equipment by a plastic tube.

5.1.3 Distribution system, consisting of a peristaltic pump allowing the sodium chloride solution (4.1) used for washing the gluten to be delivered at a constant rate of between 50 ml/min and 56 ml/min.

For a detailed description of the unit and for detailed operating instructions, users of this part of ISO 21415 should consult the leaflet of the maker of the equipment used.

5.2 Dispenser, for the sodium chloride solution, permitting delivery of 3 ml to 10 ml with an accuracy of ± 0,1 ml.

5.3 Centrifuge, capable of maintaining a rotational frequency of 6 000 ± 5 per minute and producing a radial acceleration of 2 000 *g*, fitted with 2 perforated plates with holes of diameter 600 µm and/or 2 receptacles 22 mm in diameter equipped with a grid containing 600 µm holes, to determine the gluten index (cf. Figures A.3 and A.4).

5.4 Balance, calibrated to 0,01 g.

5.5 Stainless steel spatula.

5.6 Beakers with 500 ml capacity (to catch the rinsing water).

5.7 Stainless steel or plastic grippers.

1) The Glutomatic unit (types 2100 and 2200) made by Perten Instruments AB (Sweden) is the mechanical device most widely used at present for this purpose. This information is given for the benefit of users of this part of ISO 21415 and in no way implies that ISO approves or recommends exclusive use of this device. Other equipment may also be used if it yields similar results to those of the Glutomatic unit or those of the method described in ISO 21415-1.

5.8 Lab crusher, capable of producing a ground product with a granulometry meeting the requirements of Table B.1.

5.9 Watch glass.

6 Sampling

It is important for the laboratory to receive a truly representative sample which has not been damaged or altered during transport or storage.

The method specified in this part of ISO 21415 does not cover sampling. A recommended sampling method is given in ISO 24333.

7 Preparation of test sample

Homogenize the samples. Before measuring the gluten content, grind the wheat grains and crush them with a lab crusher (5.8), as indicated in Annex B. Special precautions should be taken during grinding and storing to prevent any alteration to the moisture content of samples.

8 Procedure

8.1 General

The operations of preparation and washing of the dough are carried out continuously with automatic equipment (5.1). Follow the instructions supplied by the manufacturer of the equipment used.

8.2 Testing

Weigh 10 g of the sample to within 0,01 g for testing and transfer to the unit's washing/mixing chamber (5.1.1). Ensure that the washing chamber is fitted with a suitable sieve, which has been previously cleaned and moistened.

When testing flour samples, one or more fine polyester sieve(s) (88 µm aperture) is/are used. When testing ground wheat, chrome-plated sieve supports fitted with a slotted ring are also required with polyamide sieves with a mesh gap of 840 µm. In that case, the test starts with the fine sieve and in addition a coarse sieve is used for the second step in the method.

The gluten index is generally measured using only 88 µm polyester sieves and 840 µm polyamide sieves for the preparation of the wet gluten. If metal sieves are used, this shall be clearly noted in the test report.

8.3 Paste preparation

As a starting suggestion, add 4,8 ml of sodium chloride solution (4.1) to the test sample with the dispenser (5.2). Aim the flow of saline solution at the chamber wall so that it does not go through the sieve. Gently shake the washing chamber to ensure that the saline solution is uniformly distributed over the flour.

It may be necessary to adjust the amount of saline solution used for samples with very high or very low gluten content. If forming a consistent paste turns out to be difficult (the chamber is flooded during washing), the amount of saline solution added should be reduced (minimum of 4,2 ml). If very hard firm gluten forms during mixing, the amount of solution should be increased to 5,2 ml.

The preparation time is set by the manufacturer at 20 s, but this may be adjusted by the user, if necessary. If so, contact the manufacturer to obtain information on adjusting the regulator.

8.4 Paste washing

8.4.1 Detection of starch

For the detection of starch, press out some drops of the washing solution from the gluten ball into a watch-glass (5.9) and add a few drops of a solution of iodine (4.2) to it. If the colour of the solution does not change, the washing out procedure is completed. If the colour of the solution turns into blue, it indicates that starch is still present and the washing out procedure should be continued until the starch cannot be detected.

8.4.2 Flour

The washing time is set by the manufacturer at 5 min. A volume of 250 ml to 280 ml of sodium chloride solution is usually required for washing. The solution is delivered automatically by the equipment at a constant pre-set rate of between 50 ml/min and 56 ml/min (depending on the equipment).

8.4.3 Ground wheat

After 2 min of washing, stop the equipment, remove the washing chamber with the partially washed gluten and transfer all the content, including bran particles, to another washing chamber containing a large-grade sieve (840 µm). This can be done by placing the washing chamber beneath a gentle stream of cold water (turning one sieve round to face the other and positioning the finer sieve on top).

Place the washing chamber with the coarser sieve containing the transferred lump of gluten in the working position and continue washing until the washing sequence ends.

8.4.4 Special case

If the automatic washing process does not wash the paste sufficiently, and if the equipment allows it, repeat the test with a higher volume of sodium chloride solution.

8.5 Spinning and weighing the wet gluten

If the gluten index has to be determined, this operation is not needed, so go directly to 8.6.

When washing is complete, remove the wet gluten from the washing chamber using the grippers (5.7). Check that no gluten remains inside the washing chamber.

Divide the gluten into two equal lumps and place them on the perforated plates of the centrifuge (5.3), pressing down on them lightly.

Operate the centrifuge to remove the excess solution from the gluten (the pre-set time is 60 s). Remove the gluten piece with the metal grippers (5.7) and weigh it all (m_1) immediately to within 0,01 g (5.4).

There is no need to divide the gluten if a stabilizer is used inside the centrifuge.

If a double unit is used, two lumps of gluten will be produced. These should be treated separately one after the other.

8.6 Determining the gluten index

The extracted lump of gluten shall be centrifuged, without dividing it. Hence, two gluten measurements should be taken at the same time. This operation is possible with a double-facility unit. In case of a single-facility unit, a counterweight should be used during centrifuging.

After washing (8.4) and omitting the spin, use the grippers (5.7) to place the lump of gluten in the receptacle (5.3) provided. This is a tricky operation and shall not stretch or compress the gluten. The interval between the end of the washing cycle and the start of centrifuging should be between 20 s and 30 s. Centrifuging is programmed for 60 s.

After centrifuging, remove the receptacle and check that no gluten remains inside the centrifuge. Using the spatula (5.5), carefully scrape up all the gluten which has passed through the sieve. Weigh this to within 0,01 g (5.4). Leave this quantity on the balance and add the gluten remaining on the sieve (inside the receptacle) to estimate the total mass of wet gluten.

Only the sieve cassette (5.3) should be used for the gluten index determination.

8.7 Number of measurements

Take two measurements from the same sample.

9 Calculations and record of results

9.1 The wet gluten content (G_{hum}), expressed as a percentage by mass, is calculated with the Formula (1):

$$G_{hum} = m_1 \times 10 \qquad (1)$$

where m_1 is the mass of wet gluten (see 8.5), in grams.

The result will be the arithmetical average of two readings if the repeatability conditions (see 10.2) are observed. Express the result to within one decimal place.

9.2 The gluten index is calculated with Formula (2):

$$I_{gluten} = \frac{(m_1 - m_2)}{m_1} \times 100 \qquad (2)$$

where

m_1 is the total mass of wet gluten, in grams;

m_2 is the mass of gluten, in grams, that has passed through the sieve.

The result will be the arithmetical average of two readings if the repeatability conditions (see 10.2) are observed. Express the result to within one unit.

10 Precision

10.1 Interlaboratory tests

Details of the interlaboratory tests in terms of the method's precision are summarized in Annex C. The values obtained from these tests may not apply to concentration ranges and matrices other than those stated in the field of application.

That is, for a wet gluten content between 17,6 % and 34,7 % and a gluten index between 43 and 98.