INTERNATIONAL STANDARD

ISO 7495

First edition
1990-07-15

Wheat flour — Determination of wet gluten content by mechanical means

Farines de blé tendre — Détermination du gluten humide par des moyens mécaniques

Reference number
ISO 7495:1990(E)

Foreword

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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.

International Standard ISO 7495 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

Annex A of this International Standard is for information only.

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Printed in Switzerland

Introduction

The method of wet gluten determination specified in this International Standard requires the use of a specific apparatus. This method gives a better reproducibility but values 1,5 % to 2,5 % lower (absolute) than those obtained by using the manual extraction method specified in ISO 5531:1978, *Wheat flour — Determination of wet gluten*.

If a complementary determination of dry gluten is required, the method specified in ISO 6645:1981, *Wheat flour — Determination of dry gluten*, should be used.

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Wheat flour — Determination of wet gluten content by mechanical means

1 Scope

This International Standard specifies a method for the determination of the wet gluten content in wheat (*Triticum aestivum* L.) flour by mechanical means.

The method is applicable to commercial wheat flours and to test millings but not to coarse whole meal.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 712:1985, *Cereals and cereal products — Determination of moisture content (Routine reference method).*

ISO 2170:1980, *Cereals and pulses — Sampling of milled products.*

3 Definition

For the purposes of this International Standard, the following definition applies.

wet gluten in wheat flour: Plasto-elastic substance, composed principally of two protein fractions — gliadin and glutenin — in hydrated form, obtained using the method specified in this International Standard.

4 Principle

Preparation of a dough from a sample of wheat flour and buffered sodium chloride solution. Separation of the wet gluten by washing the dough with buffered sodium chloride solution, removal of excess solution from the gluten by centrifuging, and weighing of the residue.

5 Reagents

All the reagents used shall be of recognized analytical grade. The water used shall be distilled water or water of at least equivalent purity.

5.1 Sodium chloride, 20 g/l solution, buffered at pH 5,95.

Dissolve 200 g of sodium chloride in water and add 7,54 g of potassium dihydrogen phosphate (KH_2PO_4) and 2,46 g of sodium monohydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$). Make up to 10 l with water. Check the pH of the solution using a pH meter, and adjust if necessary.

Prepare this solution on the day of use. Before use, ensure that the solution is at a temperature of between 15 °C and 25 °C.

5.2 Iodine, solution, $c(0{,}5I_2) \approx 0{,}001$ mol/l.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Automatic apparatus for gluten separation[1], (see figure 1), comprising

a) a kneader,

1) Glutomatic 2200 is an example of a suitable apparatus available commercially. This information is given for the convenience of the users of this International Standard and does not constitute an endorsement by ISO of this apparatus.

b) a mixing/washing chamber, of 60 mm external diameter, fitted with a removable perforated metal plate having apertures of size 80 µm,

c) a dispensing device, consisting of a peristaltic pump, to deliver the sodium chloride solution (5.1) for the washing (separation) at a constant flow-rate of 50 ml/min to 54 ml/min,

d) an automatic pipette, and

e) a container, made of plastic, of 10 l capacity, for the sodium chloride solution (5.1) used in the washing (separation).

NOTE 1 For a detailed description of the apparatus and for detailed operating instructions, users of this International Standard should consult the manufacturer's manual for the apparatus employed.

6.2 Centrifuge, capable of maintaining a rotational frequency of 6 000 min^{-1} and of producing a radial acceleration of 2 000 g, equipped with perforated trays having apertures 500 µm in diameter.

6.3 Balance, accurate to 0,01 g.

7 Sampling

Sampling shall have been carried out in accordance with ISO 2170.

8 Preparation of the test sample and determination of its moisture content

Mix well the laboratory sample. Determine the moisture content of the test sample thus obtained in accordance with ISO 712.

9 Procedure

9.1 Test portion

Weigh, to the nearest 0,01 g, 10 g of the test sample (clause 8) and transfer it quantitatively into the mixing/washing chamber of the apparatus (6.1), the perforated plate of which has been previously cleaned and wetted with the sodium chloride solution (5.1).

9.2 Preparation and washing (separation) of the dough

9.2.1 General

NOTE 2 The operations of preparation and washing (separation) of the dough are carried out as a continuous process by the automatic apparatus (6.1). The preparation time is preset by the manufacturer at 20 s, but may be adjusted by the user if necessary. The washing (separation) time is also 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 (separation) operation; this solution is delivered automatically by the apparatus at a preset constant flow-rate of 50 ml/min to 54 ml/min (depending on the apparatus).

Work in accordance with the manufacturer's operating instructions for the apparatus (6.1) in use.

9.2.2 Preparation of the dough

Under normal conditions, add 4,9 ml to 5,2 ml of the sodium chloride solution (5.1) to the test portion using the automatic pipette.

For products having very high or very low gluten contents, determine by a preliminary test the volume of sodium chloride solution (5.1) necessary to prepare the dough. The minimum volume of sodium chloride solution used for dough preparation shall be 4,2 ml. Add the volume of sodium chloride solution thus determined to the test portion using the automatic pipette.

9.2.3 Washing (separation) of the dough

9.2.3.1 General case

During the washing (separation) process, observe the clarity of the effluent leaving the mixing/washing chamber. The dough is considered to have been washed sufficiently when only traces of starch are present in this effluent (i.e. the effluent is clear).

Use the iodine solution (5.2) to detect the presence of starch in the effluent.

9.2.3.2 Special case

In the case where the automatic washing process does not achieve sufficient washing of the dough carry out either of the two following operations:

a) during the washing (separation) process, add manually excess sodium chloride solution to the mixing/washing chamber;

b) regulate the apparatus to repeat the washing (separation) process.

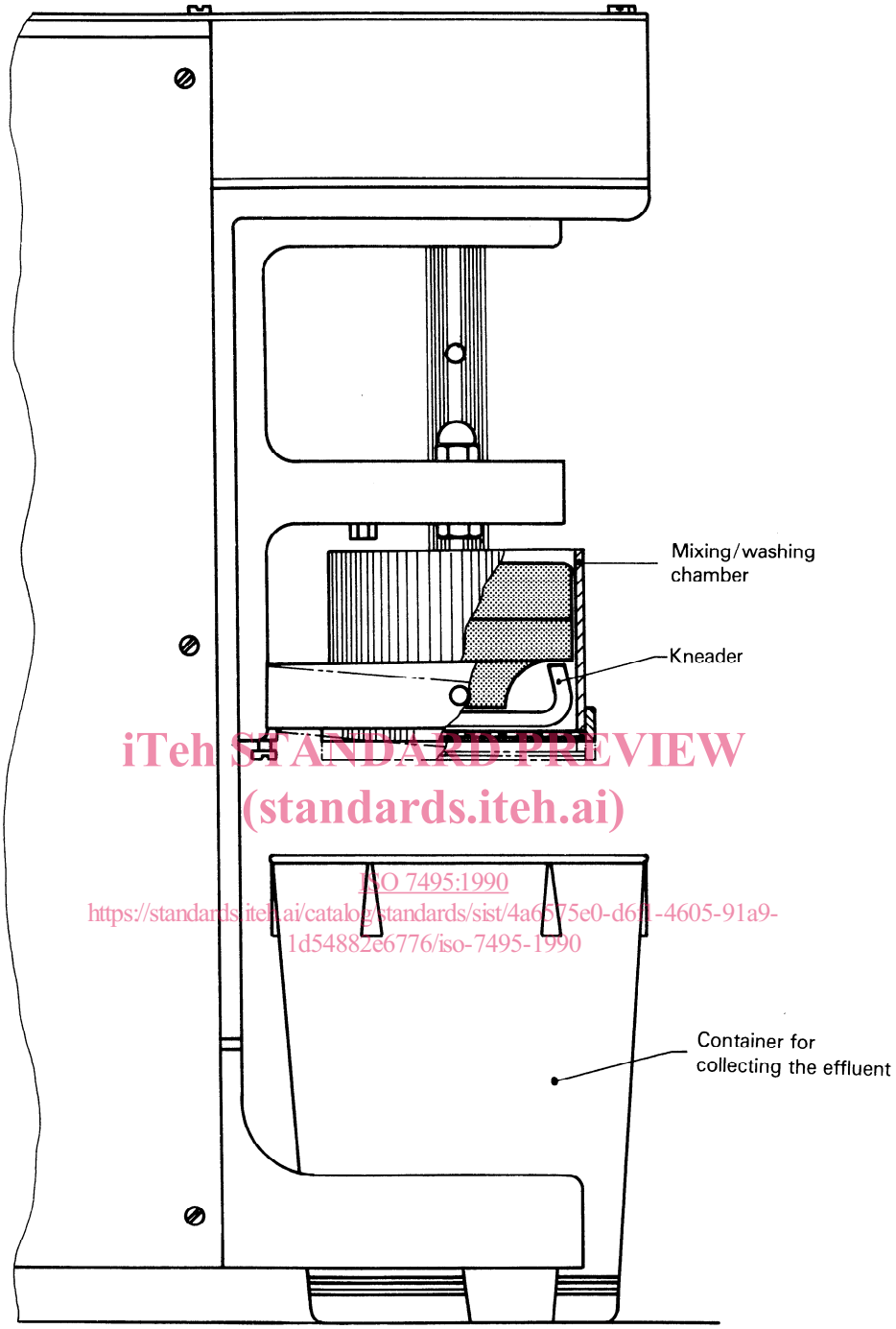

Figure 1 — Automatic apparatus for gluten separation

9.3 Centrifuging and weighing the gluten

When the washing (separation) process has been completed, remove the wet gluten from the mixing/washing chamber using forceps. Divide the gluten into two equal portions and place the balls of gluten on the perforated trays of the centrifuge (6.2), pressing them down gently.

Operate the centrifuge for 60 s to remove the excess solution from the gluten. Remove the gluten using forceps and weigh immediately to the nearest 0,01 g.

9.4 Number of determinations

Carry out two determinations on the same test sample.

10 Expression of results

The wet gluten, expressed as a percentage by mass of the product referred to a flour having a moisture content of 14 % (m/m), is equal to

$$\frac{m}{m_0} \times \frac{86}{100 - w(H_2O)} \times 100$$

where

m is the mass, in grams, of the wet gluten;

m_0 is the mass, in grams, of the test portion;

$w(H_2O)$ is the moisture content, as a percentage by mass, of the test sample.

Take as the result the arithmetic mean of the two determinations provided that the requirement for repeatability (see 11.1) is satisfied.

11 Precision

NOTE 3 Results of the inter-laboratory test are given in annex A.

11.1 Repeatability

For a gluten content within the range of 29,4 % (m/m) to 38,5 % (m/m), the difference between the results of two determinations, carried out in rapid succession (or simultaneously) by the same operator using the same apparatus on the same test sample, shall not exceed 1,0 % (absolute).

11.2 Reproducibility

For a gluten content within the range of 29,4 % (m/m) to 38,5 % (m/m), the difference between the final results obtained by two laboratories using this method for the analysis of the same laboratory sample shall not exceed 3,5 % (absolute).

12 Test report

The test report shall specify the method and the automatic apparatus for gluten separation used and the results obtained. It shall also mention all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the results.

The test report shall include all information necessary for the complete identification of the sample (e.g. the ash content of test millings).

Annex A
(informative)

Results of the inter-laboratory test

An inter-laboratory test, carried out at the international level in 1985, in which 17 laboratories participated, each of which carried out three determinations on each sample, gave the statistical results (evaluated in accordance with ISO 5725[2]) shown in table A.1.

Table A.1 — Statistical results of the inter-laboratory test

Sample	A	B	C	D
Number of laboratories retained after eliminating outliers	17	17	17	17
Mean [% (m/m)]	29,4	30,9	34,1	38,5
Standard deviation of repeatability, s_r [% (m/m)]	0,56	0,36	0,26	0,35
Coefficient of variation of repeatability	1,9 %	1,2 %	0,8 %	0,9 %
Repeatability, 2,83 s_r [% (m/m)]	1,6	1,0	0,7	1,0
Standard deviation of reproducibility, s_R [% (m/m)]	1,2	1,7	1,2	1,2
Coefficient of variation of reproducibility	4,2 %	5,6 %	3,6 %	3,2 %
Reproducibility, 2,83 s_R [% (m/m)]	3,5	4,9	3,5	3,5

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