INTERNATIONAL STANDARD

ISO 11052

First edition
1994-09-01

Durum wheat flour and semolina — Determination of yellow pigment content

Farines et semoules de blé dur — Détermination de la teneur en pigments jaunes

Reference number
ISO 11052:1994(E)

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.

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 11052 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 4, *Cereals and pulses*.

Annexes A and B of this International Standard are for information only.

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland

Printed in Switzerland

Durum wheat flour and semolina — Determination of yellow pigment content

1 Scope

This International Standard specifies a method for determination of the yellow pigment content in durum wheat flour and semolina (*Triticum durum L.*).

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the edition indicated was 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 edition of the standard 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).*

3 Definition

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

3.1 yellow pigment content: An essential quality factor of raw materials for the production of pasta, defined as the content of extractable carotenoids of the endosperm.

It is expressed as milligrams of β-carotene in 100 g of dry matter.

4 Principle

Extraction of the carotenoids at room temperature with water-saturated *n*-butanol. Then photometric evaluation of the optical density of the clear filtrate against β-carotene standard solutions.

5 Reagents

Use only reagents of recognized analytical grade and distilled or demineralized water or water of at least equivalent purity.

5.1 Water-saturated *n*-butanol

Prepare a solution of *n*-butanol and water in proportions 6:2 (*V*/*V*) and shake vigorously. Use the clear upper layer after separation of the phases.

5.2 Diethyl ether

5.3 Synthetic β-carotene, crystalline, finely crushed.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Grinder, having the following properties:

— constructed of a material that does not absorb water;

— easy to clean;

— of minimal dead space;

— capable of grinding rapidly and uniformly without heating, and avoiding as far as possible contact of the sample with the outside air.

6.2 Spectrometer, capable of operating at a wavelength of 440 nm.

6.3 Erlenmeyer flasks, of capacity 200 ml, preferably of brown glass, with ground glass stoppers.

6.4 Erlenmeyer flasks, of capacity 100 ml.

6.5 Volumetric flasks, of capacity 10 ml, narrow-necked, with ground glass stoppers.

6.6 Volumetric flasks, of capacities 100 ml and 250 ml, with ground glass stoppers.

6.7 Pipettes, of capacities 20 ml and 25 ml.

6.8 Watch glass or **Petri dish**, as cover for the funnel (6.10).

6.9 Filter paper, hard, fluted.

6.10 Funnel.

6.11 Analytical balance, capable of weighing to an accuracy of 0,001 g.

7 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 2170.

8 Preparation of test sample

Flour or semolina with a particle size smaller than 0,5 mm can be used as such. Grind semolina with a particle size greater than 0,5 mm, using the grinder (6.1).

NOTE 1 A comparative study has demonstrated that a particle size under 0,5 mm is appropriate.

9 Determination of moisture content

Determine the moisture content of the test sample in accordance with ISO 712.

10 Determination of yellow pigment

10.1 Preparation of extract

In a 200 ml Erlenmeyer flask (6.3), weigh, to the nearest 0,001 g, 10 g of the test sample, ground if necessary (see clause 8). Disperse it in 50 ml of water-saturated *n*-butanol (5.1) to give a homogeneous suspension. Shake gently in the stoppered flask several times during the first hour. Allow the suspension to stand overnight (16 h to 18 h) at room temperature.

NOTE 2 If no brown glass flasks are available, protect the stoppered flask from light.

The next morning, shake the contents again, then filter **completely** through the fluted filter paper (6.9) into a 100 ml Erlenmeyer flask (6.4). To avoid solvent loss by evaporation, place the funnel (6.10) on the flask and cover it with a watch glass or Petri dish (6.8).

10.2 Determination

Measure the optical density of the clear filtrate at 440 nm as absorbance, using the spectrometer (6.2). Use unfiltered water-saturated *n*-butanol for setting-up the instrument and for the blank determinations. Read the corresponding β-carotene content from the calibration curve (10.4).

10.3 Preparation of standard solution of β-carotene

In a 100 ml one-mark volumetric flask (6.6), weigh, to within 0,001 g, exactly 0,025 g of β-carotene. Dissolve it in diethyl ether (5.2). Make up to the mark with diethyl ether and mix carefully. Take, using a pipette (6.7), 20 ml of this homogeneous solution (= 5 mg of β-carotene) and place in a 250 ml one-mark volumetric flask (6.6). Make up to the mark with water-saturated *n*-butanol (5.1) and mix carefully. Then take, using the pipette, 25 ml of this solution and place in a 100 ml one-mark volumetric flask (6.6). Make up to the mark with the water-saturated *n*-butanol and mix carefully.

This is the standard solution. It has the following concentration:

1 ml = 0,005 mg = 5 µg β-carotene.

10.4 Preparation of calibration curve

Prepare suitable dilutions of the standard solution (10.3) with water-saturated *n*-butanol in calibrated 10 ml volumetric flasks (6.5) (e.g. from 0,5 ml to 3 ml of standard solution in 10 ml).

Measure the absorbance A, of each dilution and establish a calibration curve (β-carotene in 10 ml of solution as a function of absorbance).

11 Calculation

The yellow pigment content, w_P, expressed as milligrams of β-carotene in 100 g of dry matter, is equal to

$$w_P = \frac{5a}{100 - H}$$

where

a is the β-carotene content corresponding to the 10 ml extract (equivalent to 2 g of the test sample), in milligrams;

H is the moisture content of the test sample, expressed as a percentage by mass.

12 Precision

Results of an interlaboratory test are given in annex A.

12.1 Accuracy

Since β-carotene is used for calibration but is not present, or is only a minor constituent of the grain endosperm pigments, the resulting values may deviate by up to 5 % from the true value. However, as xanthophyl (lutein) is not readily available for calibration, this error is unavoidable and is of minor consequence.

12.2 Repeatability

For a yellow pigment content within the range of 0,651 mg/100 g to 0,754 mg/100 g, the absolute difference between two 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, should not be greater than 0,03 mg/100 g.

12.3 Reproducibility

For a yellow pigment content within the range of 0,651 mg/100 g to 0,754 mg/100 g, 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, should not be greater than 0,3 mg/100 g.

13 Test report

The test report shall specify

— the method in accordance with which sampling was carried out, if known,

— the method used,

— the test result(s) obtained, and

— if the repeatability has been checked, the final quoted result 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 test result(s).

The test report shall include all information necessary for the complete identification of the sample.

Annex A
(informative)

Results of interlaboratory test

An interlaboratory test, carried out by the International Association for Cereal Science and Technology (ICC) in 1989, in which seven laboratories participated, each of which carried out four determinations on each sample, gave the statistical results (evaluated in accordance with ISO 5725) shown in table A.1.

Table A.1

Sample	I	II	III	IV	V	VI
Number of laboratories retained after eliminating outliers	7	7	7	7	7	7
Mean content, mg/100 g d.m.[1)]	0,754	0,731	0,697	0,699	0,658	0,651
Standard deviation of repeatability S_r, mg/100 g d.m.	0,007 28	0,007 21	0,010 8	0,005 68	0,006 05	0,007 36
Coefficient of variation of repeatability, %	0,96	0,99	1,55	0,81	0,92	1,13
Repeatability, 2,83 S_r, mg/100 g d.m.	0,020	0,020	0,031	0,016	0,017	0,021
Standard deviation of reproducibility, S_r, mg/100 g d.m.	0,082 0	0,084 3	0,096 5	0,098 6	0,105 6	0,088 0
Coefficient of variation of reproducibility, %	10,83	11,53	13,84	14,24	16,05	13,52
Reproducibility, 2,83 S_r, mg/100 g d.m.	0,233	0,238	0,273	0,282	0,299	0,249
1) d.m. = dry matter						

Annex B
(informative)

Bibliography

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

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

ICS 67.060.00

Descriptors: agricultural products, plant products, food products, cereal products, durum wheat, flours (food), semolina, tests, determination, pigments, comparative tests.

Price based on 5 pages