INTERNATIONAL STANDARD

ISO 9648

First edition
1988-12-15

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION
ORGANISATION INTERNATIONALE DE NORMALISATION
МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ

Sorghum — Determination of tannin content

Sorgho — Dosage des tanins

Reference number
ISO 9648 : 1988 (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 approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 9648 was prepared by Technical Committee ISO/TC 34, *Agricultural food products.*

Printed in Switzerland

Sorghum — Determination of tannin content

1 Scope

This International Standard specifies a universal method for the determination of tannin content in sorghum grains.

It is not specific for one single type of polyphenols. Its usefulness, meanwhile, is justified by the good negative correlation observed between the metabolizable energy of sorghum grain, measured using animal experiments on cocks, and the results obtained using this method.

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 listed below. Members of IEC and ISO maintain registers of currently valid International Standards.

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

ISO 950 : 1979, *Cereals — Sampling (as grain).*

3 Principle

Extraction of tannins by shaking with dimethylformamide. After centrifuging, addition of ferric ammonium citrate and ammonia to an aliquot part of the supernatant liquid and spectrometric determination, at 525 nm, of the absorbance of the solution thus obtained. Determination of the tannin content using a calibration curve prepared using tannic acid.

4 Reagents

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

4.1 Tannic acid, 2 g/l solution.

Since the origin of tannic acid has a definite influence on the calibration curve, the use of Merck reference 773 tannic acid[1] is recommended in order to permit inter-laboratory comparison of results.

This solution can be kept for 1 week.

4.2 Ammonia, solution of 8,0 g/l NH_3.

4.3 Dimethylformamide, 75 % (V/V) solution.

Introduce 75 ml of dimethylformamide into a 100 ml volumetric flask. Dilute with water, allow to cool and make up to the mark.

WARNING — Dimethylformamide may be harmful to the health when inhaled or allowed to come into contact with the skin. It is also irritating to the eyes.

4.4 Ferric ammonium citrate, having an iron content between 17 % (m/m) and 20 % (m/m), 3,5 g/l solution, prepared 24 h before use.

Since the iron content of the citrate has an influence on the results, this content shall be respected imperatively.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Mechanical crusher, capable of producing particles which pass completely through the sieve (5.2).

5.2 Sieve, having apertures of size 0,5 mm.

5.3 Centrifuge, capable of producing a centrifugal acceleration of 3 000g (3 000 × 9,81 m/s^2).

5.4 Centrifuge tubes, with a capacity of approximately 50 ml, provided with stoppers ensuring hermetic sealing.

5.5 Mechanical stirrer, with a reciprocating motion, or **magnetic stirrer.**

5.6 Mechanical shaker for test tubes (Vortex type).

1) Merck reference 773 tannic acid is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

5.7 Spectrometer, with cells 10 mm thick, permitting measurements at 525 nm.

5.8 Pipettes, of 1 ml, 5 ml and 20 ml capacity.

5.9 Graduated pipettes, of 5 ml and 10 ml capacity.

5.10 Test tubes, 140 mm × 14 mm.

5.11 Volumetric flasks, of 20 ml capacity.

6 Sampling

Sampling shall be carried out in accordance with ISO 950.

Sorghum grains intended for determination of the tannin content may be conserved for several months, protected from the light, and should preferably be dried.

7 Preparation of the test sample

Remove any extraneous matter other than sorghum from the laboratory sample and crush the sample in the mechanical crusher (5.1) so as to reduce it to particles of a size which will pass completely through the sieve (5.2). Mix thoroughly.

The tannins in crushed products oxidize rapidly and therefore it is recommended to proceed immediately with the analysis after crushing.

NOTE — The crushed product may be conserved at most for several days, protected from the light, and should preferably be dried.

8 Procedure

8.1 Water content of the test sample

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

8.2 Test portion

Introduce about 1 g of the test sample (clause 7), weighed to the nearest 1 mg, into a centrifuge tube (5.4).

8.3 Determination

8.3.1 Using a pipette (5.8) introduce 20 ml of the dimethylformamide solution (4.3) into the centrifuge tube. Stopper the tube hermetically and stir it for 60 min ± 1 min using the stirrer (5.5). Then centrifuge for 10 min using an acceleration of 3 000*g*.

8.3.2 Remove 1 ml of the supernatant liquid (8.3.1) using a pipette (5.8) and introduce it into a test tube (5.10). Successively add 6 ml of water and 1 ml of the ammonia solution (4.2) using a pipette, and then shake for a few seconds using the shaker (5.6).

8.3.3 Remove 1 ml of the supernatant liquid (8.3.1) with a pipette (5.8) and introduce it into a test tube (5.10). Successively add 5 ml of water and 1 ml of the ferric ammonium citrate solution (4.4) using a pipette, shake for a few seconds using the shaker (5.6), then add 1 ml of the ammonia solution (4.2) using a pipette and shake again for a few seconds using the shaker (5.6).

8.3.4 Transfer the solutions obtained in 8.3.2 and 8.3.3 into measuring cells and, 10 min ± 1 min after the end of the operations carried out in 8.3.2 and 8.3.3, measure the absorbances, at 525 nm, using the spectrometer (5.7) against a water blank.

The result is the difference between the two absorbances.

8.4 Number of determinations

Carry out two determinations on test portions taken from the same test sample.

8.5 Establishment of the calibration curve

Determine the calibration curve on the day of the determination as indicated in a) to c).

a) Prepare six 20 ml volumetric flasks (5.11) and, using a graduated pipette (5.9), add to them respectively 0 ml, 1 ml, 2 ml, 3 ml, 4 ml and 5 ml of the tannic acid solution (4.1). Make up to the mark with the dimethylformamide solution (4.3). The calibration scale thus obtained corresponds to tannic acid contents of 0 mg/ml, 0,1 mg/ml, 0,2 mg/ml, 0,3 mg/ml, 0,4 mg/ml and 0,5 mg/ml respectively.

b) Pipette into test tubes (5.10) 1 ml of each of these solutions and add successively, using a pipette (5.8), 5 ml of water and 1 ml of the ferric ammonium citrate solution (4.4), and shake for a few seconds using the shaker (5.6). Then add 1 ml of ammonia solution (4.2) and shake again for a few seconds using the shaker (5.6).

Transfer the solutions thus obtained into measuring cells and after 10 min ± 1 min measure the absorbances, at 525 nm, using the spectrometer against a water blank.

c) Plot the calibration curve, using the absorbance values as the ordinate and the corresponding concentrations of tannic acid on the calibration scale [a)] as the abscissa, in milligrams per millilitre.

The curve should not pass through the origin and shall not be corrected for the zero of the scale.

9 Expression of results

The tannin content, expressed as a percentage by mass of tannic acid in relation to the dry matter, is equal to

$$\frac{2c}{m} \times \frac{100}{100 - H}$$

where

c is the tannic acid concentration, in milligrams per millilitre, of the test solution, read from the calibration curve [8.5 c)];

m is the mass, in grams, of the test portion (8.2);

H is the water content of the test sample, as a percentage by mass (8.1).

Take as the result the arithmetic mean of the two determinations provided that the requirements for repeatability, as calculated from table 1 (clause 10) using linear interpolation, are satisfied.

10 Precision

An inter-laboratory test, carried out in France, in which nine laboratories participated, the results of eight of which were retained for statistical analysis, each laboratory having carried out three determinations on each sample, gave the statistical results (evaluated in accordance with ISO 5725[1]) shown in table 1.

11 Test report

The test report shall specify the method used, the manufacturer and reference number of the tannic acid used, and the 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 result.

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

Table 1

Results expressed as a percentage by mass of tannic acid in relation to the dry matter

Sample	Argence variety	NK 121 variety	Sultan variety
Mean	0,05	0,62	1,11
Standard deviation of repeatability, s_r	0,01	0,02	0,02
Coefficient of variation of repeatability	21 %	3,3 %	1,9 %
Repeatability, 2,8 s_r	0,03	0,06	0,06
Standard deviation of reproducibility, s_R	0,02	0,03	0,07
Coefficient of variation of reproducibility	44 %	4,8 %	6,1 %
Reproducibility, 2,8 s_R	0,06	0,08	0,19

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

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UDC 633.174 : 543.8 : 547.98

Descriptors : agricultural products, grains (food), sorghum, chemical analysis, determination of content, tannins.

Price based on 3 pages