INTERNATIONAL STANDARD

ISO 14902

First edition
2001-10-15

Animal feeding stuffs — Determination of trypsin inhibitor activity of soya products

Aliments des animaux — Dosage de l'activité des inhibiteurs trypsiques des produits de soja

Reference number
ISO 14902:2001(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.ch
Web www.iso.ch

Printed in Switzerland

Contents

Page

Foreword iv

1 Scope 2

2 Normative reference 2

3 Term and definition 2

4 Principle 2

5 Reagents and materials 2

6 Apparatus 3

7 Sampling 4

8 Preparation of test sample 4

9 Procedure 4
9.1 Number of determinations 4
9.2 Sample extraction 4
9.3 Dilution of sample extract 5
9.4 Measurement of trypsin activity of working solution 5
9.5 Measurement of trypsin inhibitor activity 5

10 Calculation 6
10.1 Inhibition percentage of sample extract solutions 6
10.2 Trypsin inhibitor activity 7

11 Precision 7
11.1 Interlaboratory tests 7
11.2 Repeatability 7
11.3 Reproducibility 8

12 Test report 8

Annex A (normative) **Dilution scheme for sample extract** 9

Annex B (informative) **Results of interlaboratory test** 11

Bibliography 12

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 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 14902 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*.

Annex A forms a normative part of this International Standard. Annex B is for information only.

Animal feeding stuffs — Determination of trypsin inhibitor activity of soya products

1 Scope

This International Standard specifies a method for the determination of the trypsin inhibitor activity (TIA) of soya products.

This trypsin inhibitor activity is indicative of the degree of toasting of these products.

The detection limit of the method is 0,5 mg/g.

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

3 Term and definition

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

3.1
trypsin inhibitor activity
TIA
mass of trypsin inhibited by the procedure described in this International Standard, divided by the mass of the test sample

NOTE The trypsin inhibitor activity is expressed in milligrams per gram.

4 Principle

Trypsin inhibitors are extracted from the sample at pH 9,5.

The remaining trypsin activity is measured by adding benzoyl-L-arginine-*p*-nitroanilide (L-BAPA) as substrate. The quantity of released *p*-nitroaniline is measured spectrometrically.

5 Reagents and materials

Use only reagents of recognized analytical grade.

5.1 Water, complying with at least grade 3 in accordance with ISO 3696.

5.2 Sodium hydroxide solution, c(NaOH) = 0,01 mol/l.

5.3 Hydrochloric acid, c(HCl) = 6 mol/l.

5.4 Hydrochloric acid, c(HCl) = 1 mol/l.

5.5 Hydrochloric acid, c(HCl) = 0,1 mol/l.

5.6 Hydrochloric acid, c(HCl) = 0,001 mol/l.

5.7 Acetic acid, $c(CH_3COOH)$ = 5,3 mol/l.

5.8 Calcium chloride dihydrate, $CaCl_2 \cdot 2H_2O$.

5.9 Calcium chloride solution in hydrochloric acid.

Dissolve 735 mg of calcium chloride dihydrate (5.8) in 1 l of hydrochloric acid (5.6) and check the pH. The pH shall be 3,0 ± 0,1.

5.10 Bovine trypsin (Merck No. 24579 or equivalent).[1)]

See 9.4 for measurement of the activity. Store in the refrigerator (6.3).

5.11 Trypsin stock solution

Allow the trypsin (5.10) to reach room temperature. Dissolve 27,0 mg of trypsin in the calcium chloride solution (5.9) in a 100 ml volumetric flask (6.1) and dilute to the mark with the calcium chloride solution. This solution can be used for 5 days at most when stored in the refrigerator (6.3).

5.12 Trypsin working solution.

Pipette 5 ml of the trypsin stock solution (5.11) into a 100 ml volumetric flask (6.1) and dilute to the mark with calcium chloride solution (5.9).

5.13 Benzoyl-L-arginine-*p*-nitroanilide (L-BAPA).

5.14 Tris-(hydroxymethyl)aminomethane (Tris).

5.15 Dimethyl sulfoxide (DMSO).

5.16 Tris buffer/calcium chloride solution.

Dissolve 6,05 g of Tris (5.14) and 735 mg of calcium chloride (5.8) in 900 ml of water in a 1 l graduated measuring cylinder. Adjust the pH to 8,2 ± 0,1 with hydrochloric acid (5.3) and dilute to 1 l with water.

5.17 L-BAPA reagent.

Prepare this reagent on the day of use. Dissolve 60 mg of L-BAPA (5.13) in 1 ml of DMSO (5.15) in a 100 ml volumetric flask (6.1) and dilute to the mark with Tris buffer/calcium chloride solution (5.16).

---

1) Merck No. 24579 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.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Volumetric flasks, of capacity 100 ml.

6.2 Cuvettes, with optical path length 10 mm.

6.3 Refrigerator, controlled at a temperature of (4 ± 3) °C.

6.4 pH-meter, with an inaccuracy of 0,05 units.

6.5 Test tube mixer.

6.6 Spectrometer, suitable for measurements at a wavelength of 410 nm.

6.7 Stopwatch.

6.8 Water bath, with circulation pump, capable of being maintained at (37 ± 0,25) °C.

6.9 Grinding apparatus, provided with a 0,5 mm sieve.

6.10 Centrifuge, operating at a radial acceleration of approximately 1 500 g_n.

6.11 Centrifuge tubes.

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 6497 [5].

8 Preparation of test sample

Using the grinding apparatus (6.9), grind a representative part of sample so that heat production is minimal. Mix the ground sample thoroughly.

9 Procedure

9.1 Number of determinations

If it is required to check whether the repeatability limit (11.2) is met, carry out two single determinations in accordance with 9.2 and 9.5 under repeatability conditions.

9.2 Sample extraction

Weigh 1 g ± 0,001 g of the prepared test sample (clause 8) in a 100 ml conical flask and add 50 ml of sodium hydroxide solution (5.2). Completely suspend the sample. Adjust the pH to 9,5 ± 0,1 with hydrochloric acid (5.4 and 5.5). Rinse the electrode with as little water as possible. Close the conical flask and store overnight (15 h to 24 h) in the refrigerator (6.3). Place in the refrigerator the quantity of water needed for making up the sample extracts.

Transfer the sample extract to a 100 ml volumetric flask (6.1), dilute to the mark with water from the refrigerator and mix. Store the volumetric flask in the refrigerator. The sample extract remains stable for one day. After sedimentation for 15 min, the sample extract may be worked up further and diluted as required. Dilutions depend on the expected TIA value of the sample and are carried out with water at room temperature.

9.3 Dilution of sample extract

Estimate the TIA value of the sample and prepare three different dilutions of the sample extract on the basis of the dilution scheme in Table A.1, so that it may be expected that as a result of the TIA measurement (9.5) at least one of the three inhibition percentages obtained will be within the range of 40 % to 60 %.

If none of the three results is within this range, the estimation should be adapted and the procedure repeated.

9.4 Measurement of trypsin activity of working solution

Check the activity of each batch of trypsin (5.10). The difference between the absorbance of the working solution (5.12) and the absorbance of the blank ($A_r - A_{br}$) should be 0,380 ± 0,050. In this is not the case, check the qualiity of the trypsin (5.10). If necessary, take a fresh jar of trypsin.

Pipette into centrifuge tubes according to the following scheme:

	Blank standard ml	**Standard** ml
L-BAPA reagent (5.17)	5	5
Water (5.1)	3	3
Acetic acid (5.7)	1	0

Mix the contents of the tubes with the test tube mixer (6.5) and place the tubes in the water bath (6.8) for 10 min. Add:

	Blank standard ml	**Standard** ml
Trypsin working solution (5.12)	1	1

Mix the contents of the tubes with the test tube mixer and place the centrifuge tubes back in the water bath. After 10 min ± 5 s of incubation, add the following:

	Blank standard ml	**Standard** ml
Acetic acid (5.7)	0	1

Mix the contents of the tubes with the test tube mixer.

Centrifuge the tubes for 10 min in the centrifuge (6.10) at a radial acceleration of approximately 1 500 g_n.

Measure the absorbance of the clear solutions relative to water in the spectrometer (6.6) at 410 nm in a 10 mm cuvette (6.2).

These solutions remain stable for at least 2 h.

9.5 Measurement of trypsin inhibitor activity

Pipette into centrifuge tubes according to the following scheme.

Prepare for each dilution of sample extract (9.3) a corresponding blank solution. Sample extract solutions and corresponding blank solutions shall be dealt with simultaneously in the procedure, including centrifuging.

	Blank standard	Standard	Blank sample	Sample
	ml	ml	ml	ml
L-BAPA reagent (5.17)	5	5	5	5
Diluted sample extract (9.3)	0	0	1	1
Water (5.1)	3	3	2	2
Acetic acid (5.7)	1	0	1	0

Mix the contents of the tubes with the test tube mixer (6.5) and place the tubes in the water bath (6.8) for 10 min. Add the following:

	Blank standard	Standard	Blank sample	Sample
	ml	ml	ml	ml
Trypsin working solution (5.12)	1	1	1	1

Mix the contents of the tubes with the test tube mixer and place the centrifuge tubes back in the water bath (6.8). After 10 min ± 5 s incubation add the following:

	Blank standard	Standard	Blank sample	Sample
	ml	ml	ml	ml
Acetic acid (5.7)	0	1	0	1

Mix the contents of the tubes with the test tube mixer.

Centrifuge the tubes for 10 min in the centrifuge (6.10) at a radial acceleration of approximately 1 500 g_n.

Measure the absorbance of the clear solutions relative to water in the spectrometer (6.6) at 410 nm in a 10 mm cuvette (6.2).

These solutions remain stable for at least 2 h.

10 Calculation

10.1 Inhibition percentage of sample extract solutions

Calculate the inhibition percentage of the sample extract solutions by the equation:

$$i = \frac{(A_r - A_{br}) - (A_s - A_{bs})}{(A_r - A_{br})} \times 100\ \%$$