INTERNATIONAL STANDARD

ISO 5506

Third edition
2018-07

Soya bean products — Determination of urease activity

Produits dérivés du soja — Détermination de l'activité uréasique

Reference number
ISO 5506:2018(E)

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Published in Switzerland

Contents

Page

Foreword

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 2, *Oleaginous seeds and fruits and oilseed meals*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

This third edition cancels and replaces the second edition (ISO 5506:1988), of which it constitutes a minor revision. The dated references have been replaced with undated references and the reference ISO 5505:1986 has been corrected to ISO 5500.

Introduction

The method specified in this document is based on the property of soya bean products of being able to liberate ammoniacal nitrogen from a urea solution when they have not been sufficiently cooked.

Soya bean products — Determination of urease activity

1 Scope

This document specifies a method of determining the urease activity of products derived from soya beans. The method allows inadequate cooking of these products to be detected.

It is applicable to products having a urease activity of less than 1 mg of nitrogen per gram of product as received, under the conditions specified. For more active products, the method is applicable provided that the mass of the test portion is reduced (see 9.1).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5500, *Oilseed residues — Sampling*

ISO 5502, *Oilseed residues — Preparation of test samples*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
urease activity
amount of ammoniacal nitrogen liberated per minute under the operating conditions specified in this document

Note 1 to entry: It is expressed as milligrams of nitrogen per gram of the product as received.

4 Principle

Mixing of a ground test portion with a buffered urea solution. After keeping the mixture for 30 min at 30 °C, neutralization of the ammonia liberated, with an excess of hydrochloric acid solution, and back-titration with standard volumetric sodium hydroxide solution.

5 Reagents

All the reagents shall be of analytical quality and the water used shall be distilled water or water of equivalent purity.

5.1 Urea, buffer solution (pH 6,9 to pH 7,0).

Prepare a buffer solution by dissolving 4,45 g of disodium hydrogen phosphate dehydrate ($Na_2HPO_4.2H_2O$) and 3,40 g of potassium dihydrogen phosphate (KH_2PO_4) in water and making up to 1 000 ml.

Dissolve 30 g of urea (NH_2CONH_2) in the buffer solution. The solution thus prepared has a storage life of 1 month.

5.2 Hydrochloric acid, 0,1 mol/l solution.

5.3 Sodium hydroxide, standard volumetric solution, c(NaOH) = 0,1 mol/l.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Sieve, of 200 µm aperture size.

6.2 Apparatus for potentiometric titration, or **pH meter**, sensitive to the nearest 0,02 pH unit, with an **automatic burette** and **magnetic stirrer**.

NOTE An automatic titration apparatus allows more reproducible results to be obtained.

6.3 Titration flask.

6.4 Thermostatically controlled water-bath, capable of being controlled at 30 °C ± 0,5 °C.

6.5 Test tubes, 18 mm in diameter and 150 mm in length, fitted with a ground-in stopper.

6.6 Pipettes, of 10 ml capacity.

6.7 Grinding device, capable of grinding without significant heating (for example a ball mill).

6.8 Chronometer.

6.9 Analytical balance.

7 Sampling

Sampling shall be carried out in accordance with ISO 5500.

8 Preparation of the test sample

Preparation shall be carried out in accordance with ISO 5502.

Using the grinding device (6.7), grind 10 g of the sample for analysis to particles that pass completely through the sieve (6.1).

9 Procedure

9.1 Test portion

Transfer into a test tube (6.5) about 0,2 g of the test sample (Clause 8), weighed to the nearest 0,1 mg.

For samples of very high activity, the test portion may be reduced to 0,05 g.

It is recommended that samples having a fat content of more than a mass fraction of 10 % be defatted previously by cold extraction.

9.2 Determination

Using a pipette (6.6), add 10 ml of the buffered urea solution (5.1). Stopper the tube immediately and shake vigorously.

Place the test tube in the water-bath (6.4) at 30 °C ± 0,5 °C and keep it there for 30 min [measured with the chronometer (6.8)]. Using a pipette (6.6), immediately add 10 ml of the hydrochloric acid solution (5.2), cool rapidly to 20 °C and transfer the contents of the test tube quantitatively into the titration flask (6.3), rinsing the test tube twice with 5 ml portions of water.

Titrate immediately and rapidly with the sodium hydroxide solution (5.3) to pH 4,70, preferably using the potentiometric apparatus (6.2).

9.3 Number of determinations

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

9.4 Blank test

Introduce into a test tube (6.5) 10 ml of the buffered urea solution (5.1) and 10 ml of the hydrochloric acid solution (5.2), measured with a pipette (6.6). Rapidly add a test portion equal to that used for the main determination, weighed to the nearest 0,1 mg. Stopper the tube immediately and shake vigorously.

Place the test tube in the water-bath (6.4) at 30 °C ± 0,5 °C and keep it there for 30 min [measured with the chronometer (6.8)]. Cool to 20 °C, transfer the contents of the test tube into the titration flask (6.3) as specified in 9.2, and titrate with the sodium hydroxide solution (5.3) to pH 4,70.

10 Expression of results

10.1 Method of calculation

The urease activity, U, expressed in milligrams of nitrogen liberated per minute per gram of the product as received, is given by Formula (1):

$$U = \frac{14 \times c \times (V_0 - V_1)}{30 \times m} \quad (1)$$

where

V_0 is the volume, in millilitres, of 0,1 mol/l sodium hydroxide solution used for the blank test (9.4);

V_1 is the volume, in millilitres, of 0,1 mol/l sodium hydroxide solution used in the determination (9.2);

m is the mass, in grams, of the test portion (9.1);

c is the exact concentration, in moles per litres, of the sodium hydroxide solution used.