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Soya bean products — Determination of urease activity

Produits dérivés du soja — Détermination de l'activité uréasique

Reference number
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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 5506 was prepared by Technical Committee ISO/TC 34, *Agricultural food products.*

This second edition cancels and replaces the first edition (ISO 5506 : 1978), of which it constitutes a minor revision.

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Introduction

The method specified in this International Standard 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.

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Soya bean products — Determination of urease activity

1 Scope

This International Standard 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 applies 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 applies provided that the mass of the test portion is reduced (see note 1 to 9.1).

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 771 : 1977, *Oilseed residues — Determination of moisture and volatile matter content.*

ISO 5502 : 1983, *Oilseed residues — Preparation of test samples.*

ISO 5505 : 1986, *Oilseed residues — Sampling.*

3 Definition

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

urease activity : Amount of ammoniacal nitrogen liberated per minute under the operating conditions specified in this International Standard, 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 7,0).

Prepare a buffer solution by dissolving 4,45 g of disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) 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[1], or **pH meter,** sensitive to the nearest 0,02 pH unit, with an **automatic burette** and **magnetic stirrer**.

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).

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

6.8 Chronometer.

6.9 Analytical balance.

7 Sampling

Sampling shall be carried out in accordance with ISO 5505.

8 Preparation of the test sample

See ISO 5502 : 1983 and particularly subclause 5.4.

Using the grinding device (6.7), grind 10 g of the sample for analysis to particles which 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.

NOTES

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

2 It is recommended that samples having a fat content of more than 10 % (m/m) 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 to 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 the formula

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

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 litre, of the sodium hydroxide solution used.

Take as the result the arithmetic mean of the two determinations, if the requirement for repeatability (see 10.2) is satisfied.

Express the result to two decimal places.

NOTES

1 If a preliminary drying was carried out (see clause 8), modify the calculation accordingly.

2 If the urease activity is to be expressed in relation to the dry material, it is then equal to

$$U \times \frac{100}{100 - H}$$

where

U is the urease activity calculated using the formula above;

H is the moisture and volatile matter content of the product, as a percentage by mass, determined in accordance with ISO 771.

10.2 Repeatability

The difference between the values of two determinations, carried out in rapid succession by the same analyst using the same equipment on the same test sample, shall not exceed 10 % of the arithmetic mean value.

11 Test report

The test report shall specify the method 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.