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INTERNATIONAL STANDARD ISO 5506

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

Soya bean products — Determination of urease activity

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FOREWORD

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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.

International Standard ISO 5506 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in August 1976.

It has been approved by the member bodies of the following countries :

Australia	Hungary	Portugal
Austria	India	Romania
Bulgaria	Iran	South Africa, Rep. of
Canada	Israel	Spain
Chile	Korea, Rep. of	Turkey
Czechoslovakia	New Zealand	United Kingdom
France	Peru	Yugoslavia
Germany, F.R.	Poland	

No member body expressed disapproval of the document.

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INTERNATIONAL STANDARD ISO 5506-1978 (E)/ERRATUM

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Soya bean products — Determination of urease activity

ERRATUM

Page 1

Replace the second paragraph of clause 1 by the following :

"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 the note to 7.2)."

Soya bean products — Determination of urease activity

0 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.

1 SCOPE AND FIELD OF APPLICATION

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 is applicable to products having a urease activity of up to 30 mg of nitrogen per gram of product under the conditions specified.

2 REFERENCE

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

3 DEFINITION

For the purpose of this International Standard, the following definition applies :

urease activity : The amount of ammoniacal nitrogen liberated per minute by the product, under the specified conditions of operation, expressed as milligrams of nitrogen per gram of the product as received or related to the dry material.

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

The reagents shall be of analytical quality and the water used shall be distilled water or water of at least equivalent purity.

5.1 Buffered urea solution (pH 6,9 to 7,0)

Prepare a buffer solution by dissolving 4,45 g of disodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$) 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 N solution.

5.3 Sodium hydroxide, 0,1 N standard volumetric solution.

6 APPARATUS

Usual laboratory apparatus and in particular :

6.1 Sieve, of 200 µm mesh aperture.

6.2 Apparatus for potentiometric titration[1] or a **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 ± 0,5 °C.

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

6.6 One-mark pipette, 10 ml capacity, complying with class A of ISO 648.

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

6.8 Chronometer.

6.9 Analytical balance.

7 PROCEDURE

7.1 Preparation of test sample[2]

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

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

2) An International Standard dealing with the reduction of laboratory samples of oilseed residues to samples for analysis is in preparation.

In the case of abnormal products (for example, products with high moisture and volatile matter content), carry out a preliminary drying at the temperature of the laboratory before grinding the sample. Take the loss of mass due to this preliminary drying into acount when calculating the result.

7.2 Test portion

Transfer into a test tube (6.5) about 0,2 g of the test sample (7.1), weighed to the nearest 0,1 mg.

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

7.3 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 ± 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).

7.4 Number of determinations

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

7.5 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). Add rapidly 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 at 30 ± 0,5 °C (6.4) and keep it there for 30 min [measured with the chronometer (6.8)]. Warm to 20 °C, transfer the contents of the test tube to the titration flask (6.3) as specified in 7.3, and titrate with the sodium hydroxide solution (5.3) to pH 4,70.

8 EXPRESSION OF RESULTS

8.1 Method of calculation and formulae

8.1.1 *Activity of the product as received*

The urease activity, U, expressed in milligrams of nitrogen liberated per minute per gram of the product as received, is equal to

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

where

V_0 is the volume, in millilitres, of 0,1 N sodium hydroxide solution used for the blank test (7.5);

V_1 is the volume, in millilitres, of 0,1 N sodium hydroxide solution used in the determination (7.3);

m is the mass, in grams, of the test portion (7.2);

T is the exact normality of the sodium hydroxide solution used.

8.1.2 *Urease activity related to the dry material*

The urease activity, expressed in milligrams of nitrogen liberated per minute per gram of the product related to the dry material, is equal to

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

where

U is the urease activity calculated by the formula in 8.1.1;

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

Take as the result the arithmetic mean of the two determinations, if the requirement for repeatability (see 8.2) is satisfied.

Express the result to the nearest 0,1 mg.

NOTE — If a preliminary drying was carried out (see 7.1), modify the calculation accordingly.

8.2 Repeatability

The difference between the results of two determinations, carried out simultaneously or in rapid succession by the same analyst using the same equipment, shall not exceed 10 % of the mean value.

9 TEST REPORT

The test report shall show the method used and the result obtained, and in particular whether it is expressed in relation to the product as received or to the dry matter. It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that might have influenced the result.

The report shall include all details required for the complete identification of the sample.