
International Standard



5514

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Soya bean products — Determination of cresol red index

Produits dérivés du soja — Détermination de l'indice de rouge de crésol

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FOREWORD

ISO (the International Organization for Standardization) is a worldwide federation of national standards institutes (ISO member bodies). The work of developing International Standards is carried out through ISO technical committees. Every member body interested in a subject for which a technical committee has been set up 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.

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 5514 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in August 1976.

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

Australia	Iran	Romania
Austria	Ireland	South Africa, Rep. of
Bulgaria	Israel	Spain
Czechoslovakia	Kenya	Turkey
Egypt, Arab Rep. of	Korea, Rep. of	United Kingdom
France	New Zealand	Yugoslavia
Ghana	Poland	
Hungary	Portugal	

No member body expressed disapproval of the document.

Soya bean products — Determination of cresol red index

0 INTRODUCTION

The method specified in this International Standard is based on the property of cresol red to combine with the cationic groups in proteins, in particular soya proteins. Denaturation of the proteins increases the number of cationic groups able to react with cresol red : the quantity of cresol red combined enables the degree of cooking of the product to be assessed and reveals especially if there has been overcooking.

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a method for determination of the cresol red index permitting assessment of the degree to which products derived from soya beans have been cooked.

In particular, the cresol red index allows assessment of any overcooking.

2 REFERENCES

ISO 648, *Laboratory glassware — One-mark pipettes.*

ISO 3099, *Oilseed residues — Determination of total nitrogen content.*

ISO 3310/1, *Test sieves — Technical requirements and testing — Part 1 : Metal wire cloth.*

3 DEFINITION

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

cresol red index : The amount of cresol red bound by the product, under the specified conditions of operation, expressed in milligrams and related to 500 mg of crude protein.

4 PRINCIPLE

Mixing of a ground test portion with a standard cresol red solution. Spectrophotometric determination of the residual colouring material in the solution after a specified time by comparison with the initial standard cresol red solution.

5 REAGENTS

The reagents (apart from 5.3) shall be of analytical quality and the water used shall be distilled water or water of at least equivalent purity.

5.1 Cresol red, standard solution, 0,1 g/l.

Weigh, to the nearest 1 mg, 0,1 g of cresol red and dissolve it in 40 ml of a 0,1 N sodium hydroxide solution. Transfer quantitatively to a 1 000 ml one-mark volumetric flask and dilute to about 800 ml with water. Add 100 ml of a 1 N hydrochloric acid solution and dilute to the mark with water.

5.2 Sodium hydroxide, 0,02 N solution.

For products with a fat content greater than 10 % :

5.3 *n*-Hexane, technical grade.

6 APPARATUS

Ordinary laboratory apparatus and in particular :

6.1 Magnetic stirrer, 120 mm diameter.

6.2 Centrifuge, capable of producing an acceleration of 3 000 *g*, and equipped with polypropylene tubes.

6.3 Spectrophotometer, suitable for carrying out measurements at a wavelength of 570 nm; or, failing this, a **colorimeter** with an interference filter.

6.4 Pipettes, of capacity 1 ml and 20 ml, complying with the requirements for class A of ISO 648.

6.5 Sieve, of 200 μm mesh aperture, complying with the requirements of ISO 3310/1.

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

6.7 Analytical balance.

For products with a fat content greater than 10 % :

6.8 Microgrinder or mortar.

6.9 Equipment for removal of solvents under reduced pressure (for example a rotary evaporator).

7 PROCEDURE

7.1 Preparation of test sample¹⁾

Using the grinding device (6.6), grind 5 to 10 g of the sample for analysis to particles which pass completely through the sieve (6.5).

If the fat content exceeds 10 %, remove the fat from the product by carrying out the grinding in the presence of *n*-hexane (5.3), in either a microgrinder or a mortar (6.8). Remove most of the solvent by pouring it off. Remove the remainder under reduced pressure (see 6.9) and at a temperature not exceeding 60 °C.

7.2 Determination of crude protein content

Determine the crude protein content by the procedure specified in ISO 3099 (nitrogen content × 6,25).

7.3 Test portion

Transfer a test portion, weighed to the nearest 0,1 mg and corresponding to exactly 100,0 mg of crude protein (see 7.2), into a 50 ml conical flask.

7.4 Determination

Using a pipette (6.4), add 20,0 ml of the standard cresol red solution (5.1). Stir for 30 min by means of the magnetic stirrer (6.1). Transfer the mixture, without rinsing, into a centrifuge tube (6.2), and centrifuge it until the supernatant liquid is clear : for example, for 20 min at 3 000 *g*.

Using a pipette (6.4), take 1 ml of the clear supernatant liquid thus obtained, and place it in a small conical flask. Add 20,0 ml of the sodium hydroxide solution (5.2) with a pipette. Mix.

After 5 to 10 min, measure the absorbance (A_1), against water, of the solution thus obtained, using a spectrophotometer or colorimeter (6.3), at a wavelength of 570 nm, and a cell of 1 cm optical path length; in the same way, measure the absorbance (A_2) of the standard cresol red solution (5.1) diluted under the same conditions.

7.5 Number of determinations

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

8 EXPRESSION OF RESULTS

8.1 Method of calculation and formula

The cresol red index, expressed in milligrams of cresol red combined per 500 mg of crude protein ($N \times 6,25$), is equal to

$$\frac{A_2 - A_1}{A_2} \times 10$$

where

A_1 is the absorbance of the test solution containing residual cresol red;

A_2 is the absorbance of the diluted standard cresol red solution (5.1).

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.

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 5 % of the mean value.

9 TEST REPORT

The test report shall show the method used and the result obtained. 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.

1) The reduction of laboratory samples of oilseed residues to samples for analysis will form the subject of ISO 5502.