

INTERNATIONAL
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**Chillies and chilli oleoresins —
Determination of total capsaicinoid
content —**

Part 1:
(Spectrometric method)

ISO 7543-1:1994

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*Piments et leurs oléorésines — Détermination de la teneur en
capsaïcinoides totaux — 1994*

Partie 1: Méthode par spectrométrie



Reference number
ISO 7543-1:1994(E)

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 voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 7543-1 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 7, *Spices and condiments*.

ISO 7543 consists of the following parts, under the general title *Chillies and chilli oleoresins — Determination of total capsaicinoid content*:

- Part 1: *Spectrometric method*
- Part 2: *Method using high-performance liquid chromatography*

Chillies and chilli oleoresins — Determination of total capsaicinoid content —

Part 1: Spectrometric method

1 Scope

This part of ISO 7543 specifies a method for the determination, by a spectrometric method, of the total capsaicinoid content of whole or powdered chillies (usually *Capiscum frutescens* L.) and their oleoresins.

This method of analysis requires discoloration by carbon black. In certain cases, this discoloration cannot be achieved under the experimental conditions described. Then it is necessary to use the method of determination of capsaicinoids by high-performance liquid chromatography as described in ISO 7543-2¹⁾.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this part of ISO 7543. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this part of ISO 7543 are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 2825:1981, *Spices and condiments — Preparation of a ground sample for analysis*.

1) ISO 7543-2:1993, *Chillies and chilli oleoresins — Determination of total capsaicinoid content — Part 2: Method using high-performance liquid chromatography*.

3 Principle

Determination of the absorption of a methanolic solution of the extracts of chillies or chilli oleoresins at wavelengths of 248 nm and 296 nm.

3.1 Chillies in powder form

Extraction with tetrahydrofuran, then determination of the capsaicinoids by spectrometry, in accordance with the method described in this part of ISO 7543.

3.2 Whole chillies

Preparation by grinding the sample, then extraction with tetrahydrofuran, followed by determination of the capsaicinoids by spectrometry, in accordance with the method described in this part of ISO 7543.

3.3 Oleoresins of chillies

Dilution of the oleoresin in methanol, then determination of the capsaicinoids by spectrometry, in accordance with the method described in this part of ISO 7543.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

4.1 Carbon black, for analysis.

4.2 Methanol, spectrometric grade.

4.3 Methanol solution, obtained by mixing 70 parts by volume of methanol (4.2) with 30 parts by volume of water.

4.4 Hydrochloric acid solution, $c(\text{HCl}) = 1 \text{ mol/l}$.

4.5 Sodium hydroxide solution, freshly prepared, $c(\text{NaOH}) = 1 \text{ mol/l}$.

4.6 Tetrahydrofuran, freshly distilled or spectrometric grade.

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 One-mark volumetric flasks, of 25 ml, 100 ml and 250 ml capacity.

5.2 Spectrometer, single-beam or double-beam, suitable for measurements of absorption at wavelengths between 220 nm and 370 nm, and fitted with **quartz cells** of 1 cm optical path length.

5.3 Magnetic stirrer.

5.4 Membrane filter, with aperture size 0,45 μm .

5.5 Continuous-extraction apparatus, Soxhlet-type, with a flat-bottomed flask of 250 ml capacity and a ground neck.

5.6 Vacuum evaporator, rotary.

5.7 Water bath.

5.8 Test sieve, with aperture size 500 μm .

5.9 Analytical balance, capable of weighing to an accuracy of $\pm 0,000 \text{ g}$.

6 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 948²⁾.

7 Preparation of test sample

7.1 Chillies in powder form

7.1.1 Check that all of the powder passes through the 500 μm sieve (5.8). If this is not the case, grind the powder in accordance with ISO 2825 until the required particle size is obtained, then homogenize.

7.1.2 Weigh, to the nearest 0,01 g, about 10 g of homogenized powder (7.1.1), and transfer it quantitatively to the continuous-extraction apparatus (5.5).

7.1.3 Extract for 8 h using 100 ml of tetrahydrofuran (4.6).

Evaporate the solvent to the maximum extent possible in the rotary vacuum evaporator (5.6) under reduced pressure in a 250 ml round-bottomed flask on the water bath (5.7).

7.2 Whole chillies

7.2.1 Grind the chillies in accordance with ISO 2825 until the powder obtained passes entirely through the 500 μm sieve (5.8). Homogenize the powder after sieving.

7.2.2 Weigh, to the nearest 0,01 g, about 10 g of the homogenized powder (7.2.1), and transfer it quantitatively to the continuous-extraction apparatus (5.5).

7.2.3 Extract for 8 h using 100 ml of tetrahydrofuran (4.6).

Evaporate the solvent to the maximum extent possible in the rotary vacuum evaporator (5.6) under reduced pressure in a 250 ml round-bottomed flask on the water bath (5.7).

7.3 Oleoresins of chillies

7.3.1 Thoroughly homogenize the oleoresin.

7.3.2 Weigh, to the nearest 0,000 1 g, 0,5 g to 1 g of the homogenized oleoresin (7.3.1) in a 250 ml volumetric flask (5.1) with a ground glass stopper.

2) ISO 948:1980, *Spices and condiments — Sampling*.

8 Procedure

8.1 Preparation of test solutions

8.1.1 Chillies, whole or in powder form

To the extract obtained in 7.1.3 or in 7.2.3, add 0,05 g to 0,1 g of carbon black (4.1) so as to maintain a ratio of the order of 10 between the extract and carbon black.

Add about 90 ml of methanol solution (4.3).

Agitate on the magnetic stirrer (5.3) for 30 min. Allow the solution to stand for 5 min. Filter through the membrane filter (5.4) into a 100 ml volumetric flask (5.1). Dilute to the mark with methanol solution (4.3).

The filtrate shall be clear. Experience has shown that a light yellow colour does not affect the determination.

8.1.2 Oleoresins of chillies

To the test portion (7.3.2), add 0,05 g to 0,1 g of carbon black (4.1) so as to maintain a ratio of the order of 10 between the oleoresin and carbon black.

Add about 90 ml of methanol solution (4.3).

Agitate on the magnetic stirrer (5.3) for 30 min. Allow the solution to stand for 5 min. Filter through the membrane filter (5.4) into a 100 ml volumetric flask (5.1). Dilute to the mark with methanol solution (4.3).

The filtrate shall be clear. Experience has shown that a light yellow colour does not affect the determination.

8.2 Preparation of dilutions for spectrometric measurement

8.2.1 Transfer the following to a 25 ml volumetric flask (5.1):

- 3 ml of water,
- 2 ml of hydrochloric acid (4.4).

Dilute to the mark with methanol (4.2).

This solution is the "blank acid solution" (A).

8.2.2 Transfer the following to a 25 ml volumetric flask (5.1):

- 3 ml of water,

- 2 ml of sodium hydroxide solution (4.5).

Dilute to the mark with methanol (4.2).

This solution is the "blank alkali solution" (B).

8.2.3 Take three 25 ml volumetric flasks (5.1) and mark them **a1**, **a2** and **a3** respectively. Transfer to each flask:

- 1 ml of the filtrate obtained in 8.1.1 or 8.1.2,
- 2,7 ml of water,
- 2 ml of hydrochloric acid (4.4).

Dilute each flask to the mark with methanol (4.2).

8.2.4 Take three 25 ml volumetric flasks (5.1) and mark them **b1**, **b2** and **b3** respectively. Transfer to each flask:

- 1 ml of the filtrate obtained in 8.1.1 or 8.1.2,
- 2,7 ml of water,
- 2 ml of sodium hydroxide solution (4.5).

Dilute each flask to the mark with methanol (4.2).

8.3 Spectrometric measurements

8.3.1 For a double-beam spectrometer

Adjust the zero and the 100 % absorption with methanol solution (4.3).

Measure the blank absorbances at wavelengths of 248 nm and 296 nm by placing first the blank alkali solution (B) in the measuring cell and the blank acid solution (A) in the reference cell.

Measure the absorbances of each sample solution at wavelengths of 248 nm and 296 nm by placing the solution from flask **b1** in the measuring cell and the solution from flask **a1** in the reference cell. Then measure the absorbances with solutions from flasks **b2** and **a2**, and flasks **b3** and **a3**, respectively.

8.3.2 For a single-beam spectrometer

Adjust the zero and the 100 % absorption with methanol solution (4.3).

Re-adjust the zero of the apparatus by placing solution B in the measuring cell and then measure the absorbances of the solutions from flasks **a1**, **a2** and **a3**, respectively, at wavelengths of 248 nm and 296 nm.

Then measure the absorbances at wavelengths of 248 nm and 296 nm by placing solution **A** in the measuring cell, and then the solutions from flasks **b1**, **b2** and **b3**, respectively.

9 Calculation

9.1 Calculate the total capsaicinoid content, w_{248} , as a percentage by mass, at a wavelength of 248 nm, using the following formula:

$$w_{248} = \frac{(A_s - A_b) \times d}{314 \times m}$$

where

A_s is the absorbance of the sample solution;

A_b is the absorbance of the blank solution;

d is the dilution factor (equal to 25×100 under the operating conditions described in this part of ISO 7543);

m is the mass, in grams, of the test portion.

Carry out an additional dilution when the absorbance is greater than 0,8.

9.2 Calculate the total capsaicinoid content, w_{296} , as a percentage by mass, at a wavelength of 296 nm, using the following formula:

$$w_{296} = \frac{(A'_s - A'_b) \times d}{127 \times m}$$

where

A'_s is the absorbance of the sample solution;

A'_b is the absorbance of the blank solution;

d is the dilution factor (equal to 25×100 under the operating conditions described in this part of ISO 7543);

m is the mass, in grams, of the test portion.

Carry out an additional dilution when the absorbance is greater than 0,8.

9.3 The difference between the results of the measurements obtained at wavelengths of 248 nm and 296 nm shall not be more than 10 %.

If this is not the case, repeat the analysis.

10 Test report

The test report shall specify

— the method in accordance with which sampling was carried out, if known,

— the method used,

— the test result(s) obtained, and

— if the repeatability has been checked, the final quoted result obtained.

It shall also mention all operating details not specified in this part of ISO 7543, or regarded as optional, together with details of any incidents which may have influenced the test result(s).

The test report shall include all information necessary for the complete identification of the sample.

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