BS 4585 :
Part 18 : 1996

Methods of test for

Spices and condiments

Part 18. Determination of total capsaicinoid content of chillies and chilli oleoresins (high-performance liquid chromatographic method)

Committees responsible for this British Standard

The preparation of this British Standard was entrusted to Technical Committee AW/7, Spices and condiments, upon which the following bodies were represented:

Association of Public Analysts
Consumer Policy Committee of BSI
Department of Trade and Industry (Laboratory of the Government Chemist)
International General Produce Association
Leatherhead Food Research Association
Ministry of Agriculture, Fisheries and Food
Natural Resources Institute
Seasoning and Spice Association

This British Standard, having been prepared under the direction of the Consumer Products and Services Sector Board, was published under the authority of the Standards Board and comes into effect on 15 February 1996

Amendments issued since publication

Amd. No.	Date	Text affected

The following BSI references relate to the work on this standard:
Committee reference AW/7
Draft for comment 95/500122 DC

ISBN 0 580 25186 1

Contents

Foreword

This Part of BS 4585 has been prepared by Technical Committee AW/7.

The international standard ISO 7543-2 : 1993 was taken into account during the preparation of this Part of BS 4585. However, it was decided that the method described in ISO 7543-2, which involves the use of tetrahydrofuran, should not be adopted as a British Standard.

This British Standard calls for the use of substances that may be injurious to health if adequate precautions are not taken. It refers only to technical suitability and does not absolve the user from legal obligations relating to health and safety at any stage.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

Method

1 Scope

This Part of BS 4585 describes a method for the determination, by high-performance liquid chromatography (HPLC), of the total capsaicinoid content of whole and powdered chillies and their oleoresins.

2 References

2.1 Normative references

This Part of BS 4585 incorporates, by dated or undated reference, provisions from other publications. These normative references are made at the appropriate places in the text and the cited publications are listed on the inside back cover. For dated references, only the edition cited applies; any subsequent amendments to or revisions of the cited publication apply to this Part of BS 4585 only when incorporated in the reference by amendment or revision. For undated references, the latest edition of the cited publication applies, together with any amendments.

2.2 Informative references

This Part of BS 4585 refers to other publications that provide information or guidance. Editions of these publications current at the time of issue of this standard are listed on the inside back cover, but reference should be made to the latest editions.

3 Principle

Extraction of the capsaicinoids with methanol and determination by HPLC at a wavelength of 280 nm.

4 Reagents

4.1 *General.* Unless otherwise stated, use reagents of recognized analytical grade and water conforming to grade 3 of BS EN ISO 3696.

NOTE. Reference should be made to the safety data sheets available with each reagent.

4.2 *Methanol*, HPLC grade.

4.3 *Standard solution of capsaicinoids*, prepared as follows.

Weigh accurately 0.100 g of capsaicin (8-methyl-*n*-vanillyl-6-nonenamide) (98 % (*m/m*) crystalline) into a 100 ml one-mark volumetric flask containing about 50 ml of methanol. Stopper the flask, shake to dissolve, dilute to the mark with the methanol (**4.2**) and mix.

WARNING. Capsaicin should be handled with extreme care as it is toxic by inhalation, in contact with skin and if swallowed. It causes severe irritation.

4.4 *Working standard solution*, prepared as follows.

Transfer by safety pipette 5 ml of the standard solution (**4.3**) to a 50 ml one-mark volumetric flask. Dilute to the mark with the methanol (**4.2**) and mix.

4.5 *Elution solvent*, comprising 0.5 % (*V/V*) glacial acetic acid, 49.5 % (*V/V*) water, and 50.0 % (*V/V*) acetonitrile, prepared as follows.

Add 10 ml of glacial acetic acid to 990 ml of water. Add 1000 ml of acetonitrile. De-gas before use.

4.6 *Acid-washed sand.*

5 Apparatus

5.1 *Usual laboratory apparatus.*

5.2 *Continuous extraction apparatus* (Soxhlet type), fitted with a 100 ml flask.

5.3 *Extraction thimbles.*

5.4 *Membrane filters*, with a pore size of 0.02 µm.

5.5 *Disposable syringe*, of 1 ml capacity.

5.6 *High-performance liquid chromatograph*, equipped with a detector allowing measurements to be made at a wavelength of 280 nm, fitted to a recorder or integrator.

5.7 *Chromatographic column for HPLC*, of stainless steel, 25 cm long and with an inside diameter of 0.4 cm to 0.5 cm. Stationary phase: spherical silica with octadecyl functional group C_{18} and particle size of 5 µm maximum.

5.8 *Injection system*, comprising an injection valve with a 20 µl loop, or other system giving the same injection accuracy.

5.9 *Test sieve*, with an aperture size of 500 µm, conforming to BS 410.

5.10 *Filter paper*, Whatman no. 541 or similar.

6 Sampling

Sampling shall have been carried out in accordance with BS 4540 : Part 1.

7 Preparation of test sample

7.1 Chillies in powder form

7.1.1 Check that the laboratory sample passes through the test sieve (**5.9**). If this is not the case, grind the powder in accordance with BS 4540 : Part 2 until the required particle size is obtained.

NOTE. Sufficient material should be prepared, e.g. a total of 50 g for a moisture determination.

7.1.2 From the test sample prepared in accordance with **7.1.1**, weigh, into the extraction thimble (**5.3**), to the nearest 0.001 g, between 1g and 5 g, depending on the expected capsaicin content of the sample (record the mass), and mix with an equal volume of the acid-washed sand (**4.6**). Extract for 6 h to 8 h in the continuous extraction apparatus (**5.2**), using 80 ml of the methanol (**4.2**).

Cool the extract and filter it through a filter paper (**5.10**) into a 100 ml one-mark volumetric flask, washing the flask and the filter with the methanol. Dilute to the mark with the methanol and mix.

7.2 Whole chillies

Grind the laboratory sample in accordance with BS 4540 : Part 2 and proceed as described in **7.1.2**.

7.3 Oleoresins of chillies

Thoroughly homogenize the laboratory sample. Weigh, to the nearest 0.001 g, 1 g of the oleoresin into a 100 ml one-mark volumetric flask. Dilute to the mark with the methanol (**4.2**) and mix.

8 Procedure

8.1 Chilli extract and oleoresin solution

Using the disposable syringe (**5.5**) and a membrane filter (**5.4**), transfer 1 ml to 2 ml of the solution prepared in accordance with **7.1.2** or **7.3** to a small tube. Stopper the tube to prevent evaporation of the solvent. The test solution shall be clear. If this is not the case, refilter it through a membrane filter (**5.4**).

8.2 Determination

8.2.1 Set up the chromatograph (**5.6**) and the column (**5.7**) in accordance with the manufacturer's instructions. Using the injection system (**5.8**), inject 25 µl of the working standard solution (**4.4**) into the column. Commercial capsaicin yields four peaks: nordihydrocapsaicin (C_1), capsaicin (C_2), dihydrocapsaicin (C_3) and homodihydrocapsaicin (C_4). Record the results (peak areas). Inject a further 25 µl of the working standard solution (**4.4**). Record these results. The second set of results should not deviate from the first by more than 5 %.

8.2.2 Repeat the operations given in **8.2.1** using the methanolic extract of chilli. If the peak areas of the first set of these results deviate by more than 5% from those of the second set of results, repeat the determination. Use the mean of these results for the calculation in clause **9**.

NOTE. The determination should be repeated until the results do not deviate by more than 5 %.

8.2.3 Determine the moisture content, expressed as a percentage by mass of the test sample, in accordance with BS 4585 : Part 2.

9 Expression of results

The total capsaicinoid content, expressed as a percentage by mass of the dry matter, is given by the following formula:

$$\frac{(C_1 + C_2 + C_3 + C_4) \times c \times 100}{(S_1 + S_2 + S_3 + S_4) \times c_2} \times \frac{100}{100 - H}$$

where

C_1, C_2, C_3, and C_4 are the peak areas of nordihydrocapsaicin, capsaicin, dihydrocapsaicin and homodihydrocapsaicin of the test sample solution.

c is the concentration of the standard solution (**4.3**) (in g/100 ml);

S_1, S_2, S_3, and S_4 are the peak areas of nordihydrocapsaicin, capsaicin, dihydrocapsaicin and homodihydrocapsaicin of the standard solution (**4.4**);

c_2 is the concentration of the test sample solution (in g/100 ml) (see **7.1.2**);

H is the moisture content, expressed as a percentage by mass of the test sample, determined in accordance with BS 4585 : Part 2.

To convert percentage capsaicinoids to Scoville units, use the following formula:

$$\text{Scoville units} = \frac{\%\ \text{capsaicinoids} \times 15\ 000\ 000}{100}$$

10 Test report

The test report shall include the following information:

a) a complete identification of the sample;

b) a reference to this British Standard, i.e. BS 4585 : Part 18 : 1996;

c) the total capsaicinoid content, expressed as a percentage by mass of the dry matter and/or in Scoville units;

d) details of any incidents during the determination which may have influenced the results;

e) any operations not included in this British Standard, and any operations regarded as optional.

List of references (see clause 2)

Normative references

BSI publications

BRITISH STANDARDS INSTITUTION, London

BS 410 : 1986	*Specification for test sieves*
BS EN ISO 3696 : 1995	*Water for analytical laboratory use. Specification and test methods*
BS 4540 :	*Sampling of spices and condiments*
BS 4540 : Part 1 : 1981	*Methods of sampling*
BS 4540 : Part 2 : 1982	*Method for preparation of a ground sample for analysis*
BS 4585 :	*Methods of test for spices and condiments*
BS 4585 : Part 2 : 1982	*Determination of moisture content (entrainment method)*

Informative references

ISO publication

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION (ISO), Geneva.

(Publication is available from Customer Services, BSI.)

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

BSI — British Standards Institution

BSI is the independent national body responsible for preparing British Standards. It presents the UK view on standards in Europe and at the international level. It is incorporated by Royal Charter.

Contract requirements

A British Standard does not purport to include all the necessary provisions of a contract. Users of British Standards are responsible for their correct application.

Revisions

British Standards are updated by amendment or revision. Users of British Standards should make sure that they possess the latest amendments or editions.

Any person who finds an inaccuracy or ambiguity while using this British Standard should bring it to the attention of the Quality Manager, BSI without delay so that the matter may be investigated swiftly.

BSI offers members an individual updating service called PLUS which ensures that subscribers automatically receive the latest editions of standards.

Buying standards

Orders for all BSI, international and foreign standards publications should be addressed to Customer Services, Sales Department at Chiswick:
Tel: 0181 996 7000; Fax: 0181 996 7001.

In response to orders for international standards, it is BSI policy to supply the BSI implementation of those that have been published as British Standards, unless otherwise requested.

Information on standards

BSI provides a wide range of information on national, European and international standards through its Library, the Standardline Database, the BSI Information Technology Service (BITS) and its Technical Help to Exporters Service. Contact the Information Department at Chiswick:
Tel: 0181 996 7111; Fax: 0181 996 7048.

Subscribing members of BSI are kept up to date with standards developments and receive substantial discounts on the purchase price of standards. For details of these and other benefits contact Customer Services, Membership at Chiswick: Tel: 0181 996 7002; Fax: 0181 996 7001.

Copyright

BSI
389 Chiswick High Road
London
W4 4AL

9602 - 3 - 0.3k - DP

ISBN 0 580 25186 1

AW/7