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Rapeseed — Determination of glucosinolates content —

Part 2: Method using X-ray fluorescence spectrometry

The European Standard EN ISO 9167-2:1997 has the status of a British Standard

ICS 67.200.20

Committees responsible for this British Standard

The preparation of this British Standard was entrusted to Technical Committee AW/2, Oilseeds and residues, upon which the following bodies were represented:

British Food Manufacturing Industries Research Association
Department of Trade and Industry (Laboratory of the Government Chemist)
FOSFA International
Grain and Feed Trade Association
Institute of Biology
Ministry of Agriculture, Fisheries and Food
National Farmers' Union
National Institute of Agricultural Botany
Natural Resources Institute
Royal Society of Chemistry
Seed Crushers' and Oil Processors' Association
Tropical Growers' Association
United Kingdom Agricultural Supply Trade Association

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Contents

National foreword

This British Standard has been prepared by Technical Committee AW/2 and is the English language version of EN ISO 9167 *Rapeseed — Determination of glucosinolates content — Part 2:1997 Method using X-ray fluorescence spectrometry*, published by the European Committee for Standardization (CEN). It is identical with ISO 9167-2:1994 published by the International Organization for Standardization (ISO) and in the preparation of which the UK played a full part.

Cross-references

Publication referred to	**Corresponding British Standard**
ISO 542:1990	BS EN ISO 542:1995 *Oilseeds — Sampling*
ISO 664:1990	BS EN ISO 664:1995 *Oilseeds — Reduction of laboratory sample to test sample*
ISO 665:1977	BS EN ISO 665:1995 *Oilseeds — Determination of moisture and volatile matter content*
ISO 3696:1987	BS EN ISO 3696:1995 *Specification for water for laboratory use*
ISO 5725:1986[a]	BS 5497 *Precision of test methods* Part 1:1987 *Guide for the determination of repeatability and reproducibility for a standard test method by interlaboratory tests*
ISO 9167-1:1992	BS EN ISO 9167 *Rapeseed — Determination of glucosinolates content* Part 1:1995 *Method using high-performance liquid chromatography*

[a] ISO 5725:1986, to which informative reference is made in the text, has been superseded by ISO 5725-1:1994, ISO 5725-2:1994, ISO 5725-3:1994, ISO 5725-4:1994 and ISO 5725-6:1994 which are identical with BS ISO 5725 *Accuracy (trueness and precision) of measurement methods and results*, BS ISO 5725-1:1994 *General principles and definitions*, BS ISO 5725-2:1994 *Basic methods for the determination of repeatability and reproducibility of a standard measurement method*, BS ISO 5725-3:1994, *Intermediate measures of the precision of a standard measurement method*, BS ISO 5725-4:1994 *Basic methods for the determination of the trueness of a standard measurement method*, and BS ISO 5725-6:1994 *Use in practice of accuracy values.*

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Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, the EN ISO title page, pages 2 to 6, an inside back cover and a back cover.

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

EUROPEAN STANDARD
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English version

Rapeseed — Determination of glucosinolates content — Part 2: Method using X-ray fluorescence spectrometry

(ISO 9167-2:1994)

Graines de colza — Dosage des glucosinolates — Partie 2: Méthode par spectrométrie de fluorescence aux rayons X
(ISO 9167-2:1994)

Rapssamen — Bestimmung des Glucosinolatgehaltes — Teil 2: Verfahren mit Röntgenfluoreszenzanalyse
(ISO 9167-2:1994)

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart 36, B-1050 Brussels

Ref. No. EN ISO 9167-2:1997 E

Foreword

The text of the International Standard from Technical Committee ISO/TC 34, Agricultural food products, of the International Organization for Standardization (ISO) has been taken over as a European Standard by Technical Committee CEN/TC 307, Oilseeds, vegetable and animal fats and oils and their by-products — Methods of sampling and analysis, the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by July 1997, and conflicting national standards shall be withdrawn at the latest by July 1997.

According to the CEN/CENELEC Internal Regulations, the following countries are bound to implement this European Standard: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

NOTE Normative references to International Standards are listed in Annex ZA (normative).

Contents

1 Scope

This part of ISO 9167 specifies a rapid method for the determination of the total glucosinolate content of rapeseed using X-ray fluorescence spectrometry (XRF).

It is applicable to seeds with a normal protein content from 19 % to 23 %. For seeds with a protein content outside this range, account should be taken of the total protein content in the calculation of the total glucosinolate content (see reference [4]).

NOTE 1 ISO 9167-1 specifies a method using high-performance liquid chromatography (HPLC) which enables the content of different glucosinolates to be determined individually.

NOTE 2 When applied to immature seed or to seed that has been badly stored, for example under damp and warm conditions, the results obtained by the XRF method and by the HPLC method (ISO 9167-1) may not agree as closely as when applied to normal rapeseed. The HPLC method should be taken as the reference method.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 9167. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 9167 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 664:1990, *Oilseeds — Reduction of laboratory sample to test sample.*

ISO 665:1977, *Oilseeds — Determination of moisture and volatile matter content.*

3 Definition

For the purposes of this part of ISO 9167, the following definition applies.

3.1
total glucosinolate content

the total sulfur content minus those amounts that are bound in proteins or single glucosinolates that cannot be determined by direct reference methods, divided by the average stoichiometric number of sulfur atoms occurring in the glucosinolate fraction typical of *Brassica* species

4 Principle

Determination of total sulfur content in ground and compressed rapeseed by X-ray fluorescence spectroscopy. Calculation of the glucosinolate content by comparison with values of reference samples with a certified sulfur content.

5 Materials

5.1 Whole rapeseed reference materials, three samples, each with a certified sulfur content.

5.2 Whole rapeseed secondary reference materials, three samples, each with a sulfur content which is traceable to certified whole rapeseed reference materials (**5.1**).

5.3 Synthetic calibration samples, three samples, covering the range of sulfur content of interest.

NOTE 3 These samples may be those provided by the manufacturer of the X-ray spectrometer or prepared from powdered material.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 *Spoon*, allowing the taking of about 20 g (about 30 ml) of seed.

6.2 *Blender*, coffee-mill type of 100 cm^3 volume, 8 cm diameter and 180 W power.

6.3 *Spatulas*

6.4 *Aluminium cups* or *liquid cuvettes*, with windows of polycarbonate film.

6.5 *Hydraulic press*, or other device (*hand-press*) allowing repeatable application of a defined pressure.

6.6 *X-ray spectrometer*, of high dispersion (better than 50 eV) with vacuum equipment or helium atmosphere.

6.7 *Ventilated oven*, capable of operating at 85 °C ± 1 °C, or *microwave oven* of 650 W power, for drying seeds.

7 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 part of ISO 9167. A recommended sampling method is given in ISO 542[1].

8 Preparation of samples

8.1 Preparation of test samples

8.1.1 Reduce the laboratory sample in accordance with the method given in ISO 664.

8.1.2 If the moisture content of the seeds exceeds 10 %, reduce this to between 7 % and 9 % by drying about 40 g (60 ml) of the seeds, spread out in a thin layer, either in the microwave oven (**6.7**) for 2 min or in the ventilated oven for 75 min at 85 °C.

Allow the seeds to cool to room temperature before proceeding.

8.1.3 Carry out a parallel determination of the moisture content in accordance with the method given in ISO 665.

8.2 Preparation of reference samples

8.2.1 Treat the rapeseed reference materials (**5.1**) as described in **8.1.2** and **8.1.3**. Three examples of each reference sample are required.

8.2.2 Use these samples to calibrate the apparatus when it is first put into service and at each further calibration (see **9.3.2**).

8.2.3 Use a new series of reference samples of rapeseed (**5.1**) for each new calibration procedure.

9 Procedure

9.1 Test portion

9.1.1 Take, by means of the spoon (**6.1**), about 20 g (30 ml) of dried rapeseed, and grind them for 30 s in the grinder (**6.2**). Scrape off any meal adhering to the walls of the blender by means of the spatula (**6.3**) and grind the sample again for no more than 1 s, to ensure homogenization.

9.1.2 Fill the aluminium cup or liquid cuvette (**6.4**) with homogenized meal to the upper edge, or up to the mark specified by the manufacturer. Handle cups or cuvettes gently after filling to avoid separation of particles of different sizes.

NOTE 4 The quantity of meal put into the aluminium cups or liquid cuvettes depends only on the type of cup or cuvette used and does not influence the results of the determination. However, for each laboratory and each given apparatus, a constant quantity should be used.

9.2 Compression of the meal

In all cases, including the reference samples (**5.1**), the pressure shall be kept constant to within ± 10 %.

9.2.1 ***Aluminium cups***

Compress the meal by means of the hydraulic press (**6.5**) under a constant pressure of 1 t/cm^2.

9.2.2 ***Liquid cuvettes***

Compress the meal by means of the hand-press (**6.5**) under a pressure that reduces the volume of the meal to 35 % of the original volume.

9.3 Calibration

9.3.1 Verify, by means of the synthetic calibration samples (**5.3**), that the stability of the spectrometer corresponds to the manufacturer's specifications.

9.3.2 During routine use, verify the calibration of the spectrometer at least twice a day by means of the secondary reference samples (**5.2**), prepared according to the method given in **8.2**, and recalibrate if necessary by means of the synthetic calibration samples (**5.3**), using the reference values established for them (**9.4.2**).

NOTE 5 Calibration of the spectrometer using new reference rapeseeds (**5.1**) is recommended

- every 3 months to 4 months during routine use;
- when the apparatus has been out of use for more than 4 weeks;
- after any modification, repair or maintenance of the spectrometer.

9.4 Determination

9.4.1 Determine the intensity of the S-Kα line of the three reference samples (**5.1**) under the usual operating conditions (pressure of tube, electric current, gas flow, strength of vacuum, measurement time, etc.). Carry out each measurement on the three secondary samples prepared from each of the reference samples of seeds.

9.4.2 Determine the intensity of the S-Kα line of the three synthetic calibration samples (**5.3**) under the operating conditions specified in **9.4.1**.

9.4.3 Calculate, for each of the reference samples of rapeseed (**5.1**), the regression line relating sulfur content, S_R, of the reference sample, expressed in milligrams per gram of product, to the intensity of the S-Kα line measured in **9.4.1**.

The regression line is in the form:

$$S_R = bX_R + a$$

where

X_R is the intensity of the S-Kα line for the reference sample;

a and b are constants.

9.4.4 Calculate the sulfur content, S_C, of the synthetic calibration samples (**5.3**) according to the regression line:

$$S_C = bX_C + a$$

where

X_C is the intensity of the S-Kα line for the calibration sample.

9.4.5 Determine the intensity of the S-Kα line of each test sample (**8.1**) under the specified operating conditions.

Calculate the sulfur content, S_T, of each test sample by means of the regression line:

$S_T = bX_T + a$

where

X_T is the intensity of the S-Kα line for the test sample.

10 Expression of results

10.1 The total glucosinolate content, A, expressed in micromoles per gram of dry matter of the product (dried in accordance with **8.1.2**), is calculated from:

a) for the range of total sulfur contents below 4,93 mg/g

$$A = +\,2{,}53S_T + 0{,}768S_T^2 - 5{,}596$$

b) for the range of total sulfur contents greater than or equal to 4,93 mg/g

$$A = 13{,}724S_T - 42{,}10$$

10.2 Express the result as the arithmetic mean of two determinations made on separate sub-samples, to one decimal place.

NOTE 6 When the expression of results is demanded for a specified moisture content (usually 9 %), the result obtained as above should be corrected taking into account the moisture content measured in **8.1.3**.

11 Precision

An interlaboratory test was carried out in 1990 by 27 laboratories, in accordance with ISO 5725[2].

The precision values obtained are shown in Annex A .

11.1 Repeatability

The absolute difference between two independent test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, should not be greater than:

10 % of the arithmetic mean of the two results with a minimum value of 1 µmol/g for glucosinolate contents less than 20 µmol/g;

5 % of the arithmetic mean of the two results with a minimum value of 1 µmol/g for glucosinolate contents greater than 20 µmol/g.

11.2 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than:

20 % with a minimum value of 2 µmol/g for glucosinolate contents less than 20 µmol/g;

25 % with a minimum value of 5 µmol/g for glucosinolate contents greater than 20 µmol/g.

12 Test report

The test report shall specify

— the method used,

— the results 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 9167, or regarded as optional, together with details of any incidents which may have influenced the results.

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

Annex A (informative)
Statistical results of an interlaboratory test on rapeseed

Sample	Rapeseed A	Rapeseed B	Rapeseed C
Number of laboratories retained after eliminating outliers	21	20	21
Mean glucosinolate content, µmol/g dry matter	16,0	32,4	11,8
Standard deviation of repeatability, s_r	0,4	0,6	0,3
Coefficient of variation of repeatability, %	2,8	1,9	2,6
Repeatability, 2,83s_r	1,3	1,8	0,9
Standard deviation of reproducibility, s_R	1,2	1,8	0,7
Coefficient of variation of reproducibility, %	7,4	5,6	5,8
Reproducibility, 2,83s_R	3,4	5,2	2,0

Annex B (informative)
Bibliography

[1] ISO 542:1990, *Oilseeds — Sampling.*

[2] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.*

[3] ISO 9167-1:1992, *Rapeseed — Determination of glucosinolates content — Part 1: Method using high-performance liquid chromatography.*

[4] SCHNUG *et al., Fat Sci. Technol.*, 1991.

Annex ZA (normative)
Normative references to international publications with their relevant European publications

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

Publication	**Year**	**Title**	**EN**	**Year**
ISO 664	1990	*Oilseeds — Reduction of laboratory sample to test sample*	EN ISO 664	1995
ISO 665	1977	*Oilseeds — Determination of moisture and volatile matter content*	EN ISO 665	1995

List of references

See national foreword.

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