

Rapeseed — Determination of glucosinolates content —

Part 1: Method using high-performance liquid chromatography

ICS 67.200.20

National foreword

This British Standard is the UK implementation of EN ISO 9167-1:1995+A1:2013. It is identical to ISO 9167-1:1992, incorporating amendment 1:2013. It supersedes BS EN ISO 9167-1:1995 (dual numbered as BS 4289-9:1993), which is withdrawn.

Textual errors. When adopting the text of the International Standard the following textual errors were discovered. They have been marked in the text and have been reported to ISO in a proposal to amend the text of the International Standard.

In 4.5, paragraph 2, last line, “4.5.2.1” should be read as “4.5.2”.

In 4.8.3.2, the concentration C, should be read as 1.39×10^{-4} mol/l.

In 9.2 and in Figure 1, “desulfoglucobrassicin” (number 15) should be read as “desulfoneoglucobrassicin”.

The UK participation in its preparation was entrusted to Technical Committee AW/307, Oilseeds, animal and vegetable fats and oils and their by-products.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard, having been prepared under the direction of the Agriculture and Food Standards Policy Committee, was published under the authority of the Standards Board and comes into effect on 15 August 1993

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Amd. No.	Date	Comments
8830	November 1995	This amendment renumbers BS ISO 9167-1:1992 as BS EN ISO 9167-1:1995: Annex ZA added
	30 September 2013	Implementation of ISO amendment 1:2013 with CEN endorsement A1:2013: paragraph 1 of subclause 9.1 replaced

ICS 67.200.20

Descriptors: Agricultural products, plant products, oilseeds, rapeseeds, chemical analysis, determination of content, glucosinolate, high performance liquid chromatography

English version

Rapeseed — Determination of glucosinolates content — Part 1: Method using high-performance liquid chromatography

(ISO 9167-1:1992)

Graines de colza — Dosage des glusinolates —
Partie 1: Méthode par chromatographie liquide
à haute performance
(ISO 9167-1:1992)

Rapssamen — Bestimmung des
Glucosinolatgehaltes —
Teil 1: HPLC-Verfahren
(ISO 9167-1:1992)

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Foreword

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

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 February 1996, and conflicting national standards shall be withdrawn at the latest by February 1996.

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 United Kingdom.

Foreword to amendment A1

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2, www.iso.org/directives.

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The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 2, *Oleaginous seeds and fruits and oilseed meals*.

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1 Scope

This part of ISO 9167 specifies a method for the determination of the content of the different glucosinolates in rapeseeds (colza) using high-performance liquid chromatography.

NOTE 1 This method does not determine glucosinolates which are substituted on the glucose molecule, but these compounds are of little importance in commercial rapeseed.

NOTE 2 A rapid method for the determination of glucosinolates content using X-ray fluorescence spectrometry is the subject of ISO 9167-2.

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*.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*.

3 Principle

Extraction of glucosinolates by methanol, then purification and enzymatic desulfatation on ion-exchange resins. Determination using reversed-phase high-performance liquid chromatography (HPLC) with elution gradient and ultraviolet detection.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and water complying with grade 2 of ISO 3696.

4.1 Methanol, HPLC grade, 70 % (V/V) solution.

4.2 Sodium acetate, 0,02 mol/l at pH 4,0.

4.3 Sodium acetate, 0,2 mol/l solution.

4.4 Imidazole formate, 6 mol/l solution.

Dissolve 204 g of imidazole in 113 ml of formic acid in a 500 ml one-mark volumetric flask. Make up to the mark with water.

4.5 Internal standard, use either *sinigrin monohydrate* (potassium allylglucosinolate monohydrate, $M_r = 415,49$) (see 4.5.1) or, for rapeseed (cultivated or self-propagated) in which sinigrin is present naturally, *glucotropaeolin* (benzylglucosinolate, potassium salt, $M_r = 447,52$) (see 4.5.2).

For rapeseed with a low glucosinolate content ($< 20 \mu\text{m/g}$), reduce the internal standard concentration (1 mmol/l to 3 mmol/l) in 4.5.1 and 4.5.2.1.

4.5.1 Sinigrin monohydrate

4.5.1.1 Sinigrin monohydrate, 5 mol/l solution.

Dissolve 207,7 mg of potassium allylglucosinolate monohydrate in water in a 100 ml one-mark volumetric flask. Make up to the mark with water.

The solution thus prepared may be stored in a refrigerator at approximately 4 °C for up to a week or in a freezer at – 18 °C for a longer period.

4.5.1.2 Sinigrin monohydrate, 20 mmol/l solution.

Dissolve 831,0 mg of potassium allylglucosinolate monohydrate in water in a 100 ml one-mark volumetric flask. Make up to the mark with water.

The solution thus prepared may be stored in a refrigerator at approximately 4 °C for up to a week or in a freezer at – 18 °C for a longer period.

4.5.1.3 Purity check

Use one or more of the following three tests:

- HPLC analysis using the method specified in this part of ISO 9167;
- analysis of the intact sinigrin by HPLC (ion-pair technique);
- analysis of the desulfated and silylated sinigrin by gas chromatography.

For each test, the chromatogram shall show only one major peak representing at least 98 % of the total peak area.

Confirm the purity by determining the quantity of glucose released after hydrolysis with myrosinase (thioglucoside glucosylhydrolase, EC 3.2.3.1). Measure the glucose by enzymatic means. The use of a commercially available test kit facilitates the determination. Take into account any free glucose present; this is determined in the same way but without addition of myrosinase. The molar concentration of glucose measured should be at least 98 % of the molar concentration of the sinigrin solution tested.