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Rapeseed — Determination of glucosinolates content —

Part 1:

Method using high-performance liquid
chromatography

Graines de colza — Dosage des glucosinolates —

Partie 1: Méthode par chromatographie liquide à haute performance



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Foreword

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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 9167-1 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Sub-Committee SC 2, *Oleaginous seeds and fruits*.

ISO 9167 consists of the following parts, under the general title *Rapeseed — Determination of glucosinolates content*:

- *Part 1: Method using high-performance liquid chromatography*
- *Part 2: Method using X-ray fluorescence spectrometry*

Annex A of this part of ISO 9167 is for information only.

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Rapeseed — Determination of glucosinolates content —

Part 1:

Method using high-performance liquid chromatography

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.

NOTES

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

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 ultra-violet 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 glucohydrolase, 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.

4.5.2 Glucotropaeolin

NOTE 3 Glucotropaeolin is sometimes difficult to separate from other natural minor glucosinolates.

4.5.2.1 Glucotropaeolin, 5 mmol/l solution.

Dissolve 233,8 mg of glucotropaeolin in water in a 100 ml volumetric flask. Make up to the mark with water.

4.5.2.2 Glucotropaeolin, 20 mmol/l solution.

Dissolve 895,0 mg of glucotropaeolin in water in a 100 ml volumetric flask. Make up to the mark with water.

4.5.2.3 Purity check

Check the purity in accordance with the procedure described in 4.5.1.3.

4.5.2.4 Response factor

Verify that the response factors of glucotropaeolin, in comparison with sinigrin, correspond to those indicated in 9.2.

4.6 Mobile phases

4.6.1 Eluant A: water, purified by passing it through an activated charcoal cartridge (e.g. Norganic Millipore¹⁾ system) or water of equivalent purity.

4.6.2 Eluant B: acetonitrile, HPLC grade, 20 % (V/V) solution in purified water. The concentration may be modified in relation to the column used.

4.7 Ion-exchange resin, use either 4.7.1 or 4.7.2.

4.7.1 DEAE Sepharose CL-6B²⁾ suspension, available commercially ready for use, or an equivalent product.

4.7.2 DEAE Sephadex A25²⁾ suspension, prepared as follows.

Mix 10 g of DEAE Sephadex A25 resin (or an equivalent resin) in excess 2 mol/l acetic acid solution. Leave to settle. Add 2 mol/l acetic acid until the volume of the liquid is equal to twice the volume of the sediment.

1) Norganic Millipore system is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 9167 and does not constitute an endorsement by ISO of this product.

2) DEA Sepharose and Sephadex are examples of suitable products available commercially. This information is given for the convenience of users of this part of ISO 9167 and does not constitute an endorsement by ISO of these products.