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Oilseeds — Extraction of oil and preparation of methyl esters of triglyceride fatty acids for analysis by gas chromatography (Rapid method)

Graines oléagineuses — Extraction de l'huile et préparation des esters méthyliques d'acides gras de triglycérides pour analyse par chromatographie en phase gazeuse (Méthode rapide)

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Foreword

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The main task of technical committees is to prepare International Standards. 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.

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ISO 17059 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 2, *Oleaginous seeds and fruits and oilseed meals*.

Introduction

Chromatographic analysis of the fatty acid methyl esters (FAME) of oilseeds requires oil extraction from the oilseeds. To date, no International Standard has specified a method for extracting oil from oilseeds for FAME analysis. The methods usually performed in laboratories involve oil extraction for the determination of oil content and are tedious or time consuming[2], [3]. Consequently, the total duration and cost of the analysis of triglyceride fatty acids in oilseeds, including oil extraction, preparation and gas chromatography of the FAME are considerably increased by the oil extraction step.

This International Standard specifies a rapid and optimized method for a combined oil extraction and FAME preparation. The oil is only partially extracted from the seeds and the extracted fraction remains representative enough of the total content when the method is applied to the seeds specified in the Scope[4], [5]. The FAME are prepared according to the transesterification method described in ISO 5509 and slightly modified to be applied to iso-octane solutions of oil.

Taking into account that no reference method for oil extraction exists, the oil extraction method specified in this International Standard was compared to ISO 659[2] in an interlaboratory test[6]. Results showed very good agreement between the two methods except when applied to rapeseed with high erucic acid content. In this case, this method led to values of erucic acid content higher by approximately a mass fraction of 1 %.

Oilseeds — Extraction of oil and preparation of methyl esters of triglyceride fatty acids for analysis by gas chromatography (Rapid method)

1 Scope

This International Standard specifies a rapid method for extraction of oil and for preparation of the methyl esters of fatty acids. The methyl esters thus obtained can be used for gas chromatography.

This International Standard is applicable to the following oilseeds: rape, sunflower, soya beans, mustard, linseed.

NOTE Applying this rapid method to high erucic acid content rapeseed leads to an overestimation of erucic acid content by approximately a mass fraction of 1 %.

2 Normative references

The following referenced documents are indispensable for the application of this International Standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

ISO 5509:2000, *Animal and vegetable fats and oils — Preparation of methyl esters of fatty acids*

3 Principle

The oil is cold extracted from previously crushed grains by shaking in iso-octane. After filtration, the triglyceride fatty acids present in the iso-octane solution are transesterified with potassium hydroxide into methyl esters.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

4.1 Iso-octane (2,2,4-trimethylpentane) of chromatographic quality. See Annex A.

4.2 Anhydrous sodium sulfate.

4.3 Other reagents used for the preparation of the methyl esters are specified in ISO 5509:2000, 5.3.1 and 5.3.3.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Blade crusher, coffee grinder type.

5.2 Test tubes, of glass, of capacity 10 ml, with ground or screw type stopper and PTFE cap.

5.3 Graduated pipette, of capacity 5 ml.

5.4 Pasteur pipettes, of length 150 mm, filled with a glass wool wick and anhydrous sodium sulfate up to a height of 20 mm.

5.5 Test tubes, of glass, of capacity 5 ml, with ground or screw type stopper and PTFE cap.

5.6 Glass vial, of capacity 2 ml, with screw type stopper and PTFE cap.

6 Sampling

A representative sample should have been sent to the laboratory. It should not have 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 542[1].

7 Procedure

7.1 Preparation of the test sample

Reduce the sample in accordance with ISO 664 and crush a quantity of approximately 10 g using a blade crusher (5.1) during 15 s.

NOTE For samples that are non-homogeneous in nature, i.e. contain significant quantities of unseparable seeds (such as *Sinapis arvensis* in canola) a larger sample (25 g) may be required to ensure an accurate estimate of fatty acids.

7.2 Test portion

7.2.1 General

The crushed material test portion shall be adapted as a function of the oil content of the sample in order to permit the extraction of approximately 100 mg of oil.

7.2.2 Case of grains having an oil content exceeding 30 % by mass (rape, mustard, sunflower and linseed)

Mix the crushed material and weigh, to the nearest 0,02 g, approximately 0,40 g of it in a 10 ml test tube (5.2).

7.2.3 Case of grains having an oil content between 15 % and 30 % by mass (soya beans)

Mix the crushed material and weigh, to the nearest 0,04 g, approximately 0,80 g of it in a 10 ml test tube (5.2).

7.3 Extraction of the oil

Using a pipette (5.3), add 5 ml of iso-octane (4.1) to the tube containing the crushed material and stopper. Shake for 2 min, leave to settle or centrifuge if necessary.

If the supernatant of the extract is not clear, proceed with filtration and drying (7.4).

If the supernatant of the extract is clear, filtration and drying are not necessary and may be omitted. Transfer 3 ml of the supernatant to a 5 ml test tube (5.5). The extract is then ready for the preparation of the methyl esters (7.5).

7.4 Filtration and drying of the extract

Place the Pasteur pipette (5.4) containing anhydrous sodium sulfate (4.2) above a 5 ml test tube (5.5). Transfer the supernatant of the extract (7.3) to the Pasteur pipette and allow to drain off to obtain a volume of approximately 3 ml of clear extract in the test tube. This extract is then ready for the preparation of the methyl esters (7.5).

7.5 Preparation of the methyl esters

Proceed according to ISO 5509:2000, from 5.6.2.2 to 5.6.2.4 on the extract prepared in the 5 ml test tube (7.3 or 7.4).

NOTE The reagents are specified in ISO 5509:2000, 5.3.1 and 5.3.3.

Transfer (with dilution or not, according to ISO 5509:2000, 5.6.2.4 and the injection mode in gas chromatography) the supernatant containing the methyl esters into a 2 ml glass vial (5.6) with stopper. The solution is ready to inject into the gas chromatograph.

8 Test report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, with reference to this International Standard;

— all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s).

Annex A
(normative)

General analytical procedures

A.1 Reagents

The reagents shall not produce peaks that interfere with those of the methyl esters of fatty acids during gas-liquid chromatography.

Consequently, any new batch of reagent or solvent should be checked by using it to prepare the methyl esters of pure oleic acid and analysing them by gas-liquid chromatography. If any extra peaks appear, the reagent should be rejected.

A.2 Storage of methyl ester solution

The esters should preferably be analysed as soon as possible. If necessary, the iso-octane solution containing the methyl esters may be stored under inert gas in a refrigerator.

For a longer period of storage, it is advisable to protect the methyl esters against autoxidation by adding to the solution an antioxidant in such a concentration as will not interfere with the subsequent analysis, for example a 0,05 g/l solution of BHT (2,6-di-*t*-butyl-4-methylphenol).

Bibliography

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

[2] ISO 659:1998, *Oilseeds — Determination of oil content (Reference method)*

[3] XP V 03-030:1991 (French standard), *Agricultural products and foodstuffs — Extraction of the crude fat with a view to its characterization*

[4] BLIN, F, 1998. *Rapid extraction of oil from oilseeds with a view to the analysis of fatty acids*. Report-CETIOM

[5] QUINSAC, A. and BLIN, F. Validation of a rapid method to extract oil in oleaginous seeds for fatty acid analysis. *Proceedings of the 10th International Rapeseed Congress*, Camberra, Australia, September 26 to 29, 1999, 122

[6] CETIOM, 2002, *Report of the Ring-Test (January - April 2001): Comparison between ISO/WD 17059 and ISO 659 – ISO 5509*

[7] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[8] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*