INTERNATIONAL STANDARD

ISO 17059

Second edition
2019-07

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)

Reference number
ISO 17059:2019(E)

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Published in Switzerland

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Foreword

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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 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

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

This second edition cancels and replaces the first edition (ISO 17059:2007), which has been technically revised. The main changes compared with the previous edition are as follows:

— the description of the preparation of methyl esters in 8.5 has been updated to remove the reference to ISO 5509:2000, which has been withdrawn.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Chromatographic analysis of the fatty acid methyl esters (FAME) of oilseeds requires oil extraction from the oilseeds. 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 document 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 5509:2000[1)] 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 document 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 %.

1) Withdrawn standard. Replaced by ISO 12966-2:2011 and ISO 12966-3:2009.

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

1 Scope

This document 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 document is applicable to the following oilseeds: rape and mustard with low erucic acid content (< 2 %), sunflower, soya beans, 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 %. This difference was observed in Reference [6] and could be due to the partial extraction of the oil from the sample (yield around 70 %). High content of erucic acid in triglycerides could increase their solubility in hexane because of the lipophilic effect of the carbon long-chain (C22). However, as this effect was not checked on a large set of high erucic rapeseed samples, it is not appropriate to apply a correction factor to the erucic acid content when analysing high erucic acid rapeseed.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. 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 12966-4, *Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters — Part 4: Determination by capillary gas chromatography*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

4 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.

5 Reagents

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

5.1 Iso-octane (2,2,4-trimethylpentane) of chromatographic quality, in accordance with A.1.

5.2 Anhydrous sodium sulfate.

5.3 Potassium hydroxide, methanolic solution, approximately 2 mol/l. Since potassium hydroxide in practice contains about 15 % water, proceed as follows. Dissolve with gentle heating, 13,1 g of potassium hydroxide in 100 ml of absolute methanol. Add sufficient anhydrous sodium sulfate to the solution to dry it. Filter to obtain a clear solution. If the solution has to be stored for a considerable time, a small amount of white precipitate of sodium carbonate may be formed; this has no effect on the preparation of the methyl esters when using the clear supernatant.

5.4 Sodium hydrogen sulfate monohydrate.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Blade crusher, coffee grinder type.

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

6.3 Graduated pipette, of capacity 5 ml.

6.4 Pipette or automatic pipette, of capacity 200 µl.

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

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

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

7 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 document. A recommended sampling method is given in ISO 21294.

8 Procedure

8.1 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 (6.1) 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) could be required to ensure an accurate estimate of fatty acids.

8.2 Test portion

8.2.1 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.

8.2.2 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 (6.2).

8.2.3 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 (6.2).

8.3 Extraction of the oil

Using a pipette (6.3), add 5 ml of iso-octane (5.1) 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 (see 8.4).

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 (6.6). The extract is then ready for the preparation of the methyl esters (see 8.5).

8.4 Filtration and drying of the extract

Place the Pasteur pipette (6.5) containing anhydrous sodium sulfate (5.2) above a 5 ml test tube (6.6). Transfer the supernatant of the extract (see 8.3) 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 (see 8.5).

8.5 Preparation of the methyl esters

8.5.1 Proceed on the extract prepared in the 5 ml test tube (see 8.3 or 8.4). Add, with a pipette (6.4), 200 µl methanolic potassium hydroxide solution (5.3) and stopper the test tube. Shake the mixture vigorously for about 30 s. After an initial cloudiness due to the separation of glycerol, the reaction mixture will become clear.

8.5.2 Add around 1 g of sodium hydrogen sulfate monohydrate (5.4) to the solution and shake it vigorously to neutralize the potassium hydroxide.

8.5.3 After the salt has settled, decant the upper layer containing the methyl esters into the 5 ml test tube (6.6). The isooctane solution obtained will contain about 15 mg/ml of methyl esters and may be prepared for injection into the gas chromatograph according to the injection mode and the type of column, as follows:

a) without dilution for injection onto a packed column for gas-liquid chromatography;

b) after appropriate dilution with isooctane for capillary column systems prior to the injection;

c) after dilution with a lower boiling solvent such as heptane for the special case of capillary on-column injection.

Transfer the supernatant containing the methyl esters into a 2 ml glass vial (6.7) with stopper. Then inject into the gas chromatograph in accordance with ISO 12966-4. The methyl ester solution shall be stored as described in A.2.

9 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 document, i.e. ISO 17059;

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

— the date of the test.

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).