

Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters - Part 3: Preparation of methyl esters using trimethylsulfonium hydroxide (TMSH) (ISO 12966-3:2016)

## EESTI STANDARDI EESSÕNA

## NATIONAL FOREWORD

See Eesti standard EVS-EN ISO 12966-3:2016 sisaldab Euroopa standardi EN ISO 12966-3:2016 ingliskeelset teksti.	This Estonian standard EVS-EN ISO 12966-3:2016 consists of the English text of the European standard EN ISO 12966-3:2016.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 08.06.2016.	Date of Availability of the European standard is 08.06.2016.
Standard on kättesaadav Eesti Standardikeskusest.	The standard is available from the Estonian Centre for Standardisation.

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English Version

Animal and vegetable fats and oils - Gas chromatography  
of fatty acid methyl esters - Part 3: Preparation of methyl  
esters using trimethylsulfonium hydroxide (TMSH) (ISO  
12966-3:2016)

Corps gras d'origines animale et végétale -  
Chromatographie en phase gazeuse des esters  
méthyliques d'acides gras - Partie 3: Préparation des  
esters méthyliques à l'aide d'hydroxyde de  
triméthylsulfonium (TMSH) (ISO 12966-3:2016)

Tierische und pflanzliche Fette und Öle -  
Gaschromatographie von Fettsäuremethylestern - Teil  
3: Herstellung von Methylestern mittels  
Trimethylsulfoniumhydroxid (TMSH) (ISO 12966-  
3:2016)

This European Standard was approved by CEN on 12 May 2016.

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EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

## European foreword

This document (EN ISO 12966-3:2016) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with 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of an identical text or by endorsement, at the latest by December 2016, and conflicting national standards shall be withdrawn at the latest by December 2016.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 12966-3:2009.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

### Endorsement notice

The text of ISO 12966-3:2016 has been approved by CEN as EN ISO 12966-3:2016 without any modification.

# Contents

	Page
Foreword.....	iv
1 Scope.....	1
2 Normative references.....	1
3 Principle.....	1
4 Reagents.....	1
5 Apparatus.....	2
6 Sampling.....	2
7 Preparation of the test sample.....	2
8 Procedure.....	2
9 Test report.....	3
Bibliography.....	4

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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](http://www.iso.org/directives)).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This second edition cancels and replaces the first edition (ISO 12966-3:2009), of which it constitutes a minor revision. The scope has been revised to state that the document is not applicable to milk and milk fat products.

ISO 12966 consists of the following part, under the general title *Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters*:

- *Part 1: Guidelines on modern gas chromatography of fatty acid methyl esters*
- *Part 2: Preparation of methyl esters of fatty acids*
- *Part 3: Preparation of methyl esters using trimethylsulfonium hydroxide (TMSH)*
- *Part 4: Determination by capillary gas chromatography*

# Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters —

## Part 3:

## Preparation of methyl esters using trimethylsulfonium hydroxide (TMSH)

### 1 Scope

This part of ISO 12966 specifies a rapid base-catalysed transesterification method for fats and oils with trimethylsulfonium hydroxide (TMSH) to prepare fatty acid methyl esters. The method is exclusively applicable to the preparation of methyl esters of fats and oils for gas liquid chromatographic (GLC) analysis. It is applicable to all fats and oils, but excluding those coming from milk and milk products. Isomerization of unsaturated fatty acids only occurs to a minor extent and isomerized fatty acids are only present at the determination limit. As isomerization takes place, the procedure is not recommended for conjugated linoleic acid (CLA).

Only about 70 % to 80 % of the free fatty acids are esterified. In the case of conjugated cyclopropyl and cyclopropenyl fatty acids, side reactions may occur, but these do not interfere with the determination of the fatty acids.

NOTE This part of ISO 12966 is based upon German Standard Method C-VI 11e (98) (see Reference [8]).

### 2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 661, *Animal and vegetable fats and oils — Preparation of test sample*

### 3 Principle

The sample is dissolved in *tert*-butyl methyl ether (TBME) and mixed with a methanolic solution of trimethylsulfonium hydroxide. Glycerides are base-catalysed transesterified and fatty acid methyl esters are formed (see References [4] to [8]). Free fatty acids are converted to salts which are pyrolysed to methyl esters and dimethylsulfide in the injector. Excess reagent is also pyrolysed into methanol and dimethylsulfide. To obtain a complete pyrolytic reaction, a hot injector (split injection) of at least 250 °C is necessary.

For the determination of short-chain fatty acids (C<sub>4</sub> to C<sub>8</sub>), valeric acid methyl ester is used as an internal standard. Lipids containing hydroxy groups are partially converted to the corresponding *O*-methyl ether derivatives which may interfere with fatty acid methyl esters in the GLC separation (Reference [9]). In the early part of the chromatogram (region of C<sub>4</sub>), peaks may occur, which are from the reagent. These peaks are not taken into account.

### 4 Reagents

**WARNING** — Attention is drawn to the regulations which specify the handling of hazardous substances. Technical, organizational, and personal safety measures shall be followed.