

SLOVENSKI STANDARD

SIST EN ISO 12966-2:2011

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Nadomešča:
SIST EN ISO 5509:2001

Rastlinske in živalske maščobe in olja - Plinska kromatografija metilnih estrov maščobnih kislin - 2. del: Priprava metilnih estrov maščobnih kislin (ISO 12966-2:2011)

Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters - Part 2: Preparation of methyl esters of fatty acids (ISO 12966-2:2011)

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Tierische und pflanzliche Fette und Öle - Gaschromatographie von Fettsäuremethylestern - Teil 2: Herstellung von Fettsäuremethylestern (ISO 12966-2:2011)

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Corps gras d'origines animale et végétale - Chromatographie en phase gazeuse des esters méthyliques d'acides gras - Partie 2: Préparation des esters méthyliques d'acides gras (ISO 12966-2:2011)

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71.040.50	Fizikalnokemijske analitske metode	Physicochemical methods of analysis

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EUROPEAN STANDARD

EN ISO 12966-2

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Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters - Part 2: Preparation of methyl esters of fatty acids (ISO 12966-2:2011)

Corps gras d'origines animale et végétale -
Chromatographie en phase gazeuse des esters
méthylliques d'acides gras - Partie 2: Préparation des
esters méthylliques d'acides gras (ISO 12966-2:2011)

Tierische und pflanzliche Fette und Öle -
Gaschromatographie von Fettsäuremethylestern - Teil 2:
Herstellung von Fettsäuremethylestern (ISO 12966-2:2011)

This European Standard was approved by CEN on 14 February 2011.

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Foreword

This document (EN ISO 12966-2:2011) 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of an identical text or by endorsement, at the latest by August 2011, and conflicting national standards shall be withdrawn at the latest by August 2011.

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.

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INTERNATIONAL STANDARD

ISO
12966-2

First edition
2011-02-15

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

Part 2: Preparation of methyl esters of fatty acids

*Corps gras d'origines animale et végétale — Chromatographie en phase gazeuse des esters méthyliques d'acides gras —
Partie 2: Préparation des esters méthyliques d'acides gras*

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ISO 12966-2:2011(E)

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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.

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.

ISO 12966-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This first edition of ISO 12966-2 cancels and replaces ISO 5509:2000, of which it constitutes a technical revision.

ISO 12966 consists of the following parts, under the general title *Animal and vegetable fats and oils — 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)*

The following part is under preparation:

- *Part 4: Determination of cis-, trans-, saturated, mono- and polyunsaturated fatty acids in vegetable or non-ruminant oils and fats*

The following part is planned:

- *Part 1: Guidelines on gas chromatography*

Introduction

General

Oils and fats (i.e. liquid and solid lipids) are predominantly composed of fatty acid esters of glycerol (triacylglycerols, TAGs), with smaller amounts of fatty acid esters of sterols and long chain aliphatic alcohols. Due to the high molecular mass of the TAGs and their consequent low volatility, they are difficult to analyse directly by gas chromatography (GC), especially if a detailed analysis of unsaturated fatty acids is required. Fatty acids themselves do not chromatograph well (except for short-chain-length fatty acids, e.g. butanoic and pentanoic acids). It is therefore better practice to form fatty acid esters, usually the fatty acid methyl esters (FAMES), prior to GC.

The analysis of oils and fats has been extensively reviewed in Reference [9].

The formation of FAMES is a critical stage in the analysis of fatty acids. Non-quantitative conversion of fatty acids to FAMES, modification of the structure of fatty acids (e.g. changes in positional and geometric isomers present) and formation of non-FAME artefacts may all affect the quantitative determination of fatty acid composition.

Transesterification is one mechanism which can be employed to form FAMES from fatty acid esters in fats (i.e. triacylglycerol). Alkali- or acid-catalysed transesterification procedures can be used to form FAMES in a methanolic medium; the procedure can be termed *transmethylation*. Transmethylation is a reversible process and a large excess of methanol is required to maintain an equilibrium position which favours formation of the FAMES. Water can prevent the reaction going to completion, and its presence should therefore be minimized. Alkali-catalysed procedures do not produce FAMES from free fatty acids, due to the formation of soaps.

Esterification is an acid-catalysed mechanism which can be employed to form FAMES from fatty acids. It is possible that the fatty acids are naturally present in the sample of fat under examination. Formation of FAMES by this mechanism is commonly termed *methylation*. Again, an excess of methanol and the absence of water are preconditions for the quantitative formation of FAMES.

This part of ISO 12966 provides guidelines for the preparation of fatty acid methyl esters. In support of these guidelines, various procedures to prepare fatty acid methyl esters are specified. These include:

- a) "rapid" transmethylation under alkaline conditions;
- b) "general" transmethylation/methylation under sequential alkaline and acid conditions;
- c) boron trifluoride (BF₃) transmethylation/methylation.

"Rapid" transmethylation method under alkali-catalysed conditions

This method is applicable to the routine analysis of edible fats and oils containing fatty acids down to butanoic acid (C4:0) and/or for the determination of butanoic acid or hexanoic acid (C6:0) by GC using an internal standard.

Alkaline catalysts transesterify neutral lipids in the presence of anhydrous methanol (transmethylation) more rapidly than acid catalysts. The disadvantages of such alkali-catalysed procedures are that free fatty acids are not esterified, and the presence of water may prevent the transmethylation going to completion (hydrolysis of the FAMES to free fatty acids). The most commonly used reagents are potassium and sodium hydroxide and sodium methoxide in the presence of anhydrous methanol.