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Rastlinske in živalske maščobe in olja - Plinska kromatografija metilnih estrov maščobnih kislin - 2. del: Priprava metilnih estrov maščobnih kislin (ISO 12966-2:2017)

Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters - Part 2: Preparation of methyl esters of fatty acids (ISO 12966-2:2017)

Tierische und pflanzliche Fette und Öle - Gaschromatographie von Fettsäuremethylestern - Teil 2: Herstellung von Fettsäuremethylestern (ISO 12966-2:2017)

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 12966-2:2017)

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Tierische und pflanzliche Fette und Öle - Gaschromatographie von Fettsäuremethylestern - Teil 2: Herstellung von Fettsäuremethylestern (ISO 12966-2:2017)

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European foreword

This document (EN ISO 12966-2:2017) 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of an identical text or by endorsement, at the latest by October 2017 and conflicting national standards shall be withdrawn at the latest by October 2017.

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Endorsement notice

The text of ISO 12966-2:2017 has been approved by CEN as EN ISO 12966-2:2017 without any modification.

INTERNATIONAL STANDARD

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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

Corps gras d'origines animale et végétale — Chromatographie en phase gazeuse des esters méthyliques d'acides gras —

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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).

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

This second edition cancels and replaces the first edition (ISO 12966-2:2011), of which it constitutes a minor revision.

The changes compared to the previous edition are as follows:

— milk and milk fat products have been excluded from the scope.

A list of all the parts in the ISO 12966 series can be found on the ISO website.

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 of 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 document 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 the following:

a) “rapid” transmethylation under alkaline conditions;

b) “general” transmethylation/methylation under sequential alkaline and acid conditions;

c) boron trifluoride (BF_3) 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.

“General” transmethylation/methylation under sequential alkaline and acid conditions

This method under sequential alkali- and acid-catalysed conditions is applicable to all oils and fats including distillate and acid oils, but is not recommended for lauric oils. Short-chain fatty acid methyl esters are easily lost during reflux. For lauric acid oils, the “rapid” transmethylation method is recommended.