



**International
Standard**

ISO 12966-4

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

**Part 4:
Determination by capillary gas
chromatography**

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

*Partie 4: Détermination par chromatographie capillaire en phase
gazeuse*

**Second edition
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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 document 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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

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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 11, *Animal and vegetable fats and oils*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 307, *Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 12966-4:2015), which has been technically revised.

The main changes are as follows:

- the Scope has been extended to the separation of fatty acid methyl esters from C4 to C24;
- ruminant fat has been added to the Scope,
- quantification by area (%) or by mass (g/100 g) using internal standards and correction factors calculated with a quantitative fatty acid methyl esters standard mixture containing *cis* and *trans* fatty acid methyl esters from C4:0 to C22:6; has been added
- quantification of total *trans* fatty acid methyl esters by mass (g/100 g) has been added;
- the use of 100 m, 0,25 mm ID, 0,20 µm film thickness columns are now required to separate most C18:1 *trans*- and *cis*-isomers;
- a method has been added for determination of the composition of fatty acid methyl esters expressed by area % in liquid vegetable oils.

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

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

This document is one part of a series of four International Standards for the preparation and determination of fatty acid methyl esters (FAMES) by gas chromatography in animal and vegetable fats and oils. The ISO 12966 series is applicable to crude, refined, partially hydrogenated, or fully hydrogenated fats, oils, and fatty acids derived from animal and vegetable sources, and fats extracted from foodstuff.

The ISO 12966 series is not suitable for milk and milk products (or fat coming from milk and milk products), or products supplemented with conjugated linoleic acid (CLA). Furthermore, it is not intended to be applied to polymerized and oxidized fats and oils.

This document gives the conditions for the analysis of FAMES by capillary gas chromatography, while ISO 12966-2 and ISO 12966-3 cover the preparation of FAMES by different methods. ISO 12966-1 is a guideline to the modern gas chromatography of FAMES.

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

Part 4: Determination by capillary gas chromatography

1 Scope

This document specifies a method for the determination of fatty acid methyl esters (FAMES) derived by transesterification or esterification from fats, oils, and fatty acids by capillary gas chromatography (GLC). FAMES from C4 to C24 can be separated using this document including saturated FAMES, *cis*- and *trans*-monounsaturated FAMES, and *cis*- and *trans*-polyunsaturated FAMES.

This document is applicable to crude, refined, partially hydrogenated or fully hydrogenated fats, oils and fatty acids derived from animal and vegetable sources, and fats extracted from foodstuff.

This document does not apply to milk and milk products (or fat coming from milk and milk products) or products supplemented with conjugated linoleic acid (CLA).

This document does not apply to di-, tri-, polymerized, hydroxylated and oxidized fatty acids, and fats and oils.

A method for the determination of the composition of FAMES expressed by area % in liquid vegetable oils is proposed in [Annex E](#).

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

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 6353-2, *Reagents for chemical analysis — Part 2: Specifications — First series*

ISO 6353-3, *Reagents for chemical analysis — Part 3: Specifications — Second series*

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

3 Terms and definitions

No terms and definitions are listed in this document.

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

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