



**SLOVENSKI STANDARD**  
**SIST EN ISO 5509:2001**

01-februar-2001

Animal and vegetable fats and oils - Preparation of methyl esters of fatty acids (ISO 5509:2000)

Tierische und pflanzliche Fette und Öle - Herstellung von Fettsäuremethylestern (ISO 5509:2000)

Corps gras d'origines animale et végétale - Préparation des esters méthyliques d'acides gras (ISO 5509:2000)

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**ICS:**

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EUROPEAN STANDARD  
NORME EUROPÉENNE  
EUROPÄISCHE NORM

EN ISO 5509

April 2000

ICS 67.200

English version

Animal and vegetable fats and oils - Preparation of methyl esters  
of fatty acids (ISO 5509:2000)

Corps gras d'origines animale et végétale - Préparation des  
esters méthyliques d'acides gras (ISO 5509:2000)

Tierische und pflanzliche Fette und Öle - Herstellung von  
Fettsäuremethylestern (ISO 5509:2000)

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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

Central Secretariat: rue de Stassart, 36 B-1050 Brussels

## Foreword

The text of the International Standard ISO 5509:2000 has been prepared by Technical Committee ISO/TC 34 "Agricultural 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of an identical text or by endorsement, at the latest by October 2000, and conflicting national standards shall be withdrawn at the latest by October 2000.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

**NOTE FROM CEN/CS:** The foreword is susceptible to be amended on reception of the German language version. The confirmed or amended foreword, and when appropriate, the normative annex ZA for the references to international publications with their relevant European publications will be circulated with the German version.

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**Endorsement notice**

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The text of the International Standard ISO 5509:2000 was approved by CEN as a European Standard without any modification.

**NOTE:** Normative references to International Standards are listed in annex ZA (normative).

**Annex ZA (normative)**  
**Normative references to international publications**  
**with their relevant European publications**

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

<u>Publication</u>	<u>Year</u>	<u>Title</u>	<u>EN</u>	<u>Year</u>
ISO 661	1989	Animal and vegetable fats and oils - Preparation of test sample	EN ISO 661	1995
ISO 3696	1987	Water for analytical laboratory use - Specification and test methods	EN ISO 3696	1995

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# INTERNATIONAL STANDARD

**ISO**  
**5509**

Second edition  
2000-04-01

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## **Animal and vegetable fats and oils — Preparation of methyl esters of fatty acids**

*Corps gras d'origines animale et végétale — Préparation des esters  
méthyliques d'acides gras*

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Reference number  
ISO 5509:2000(E)

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 5509 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This second edition cancels and replaces the first edition (ISO 5509:1978), which has been technically revised.

Annex A forms a normative part of this International Standard. Annex B is for information only.

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**ISO 5509:2000(E)****Introduction**

ISO 5509 contains three different procedures to prepare methyl esters.

The general method is the  $\text{BF}_3$  method which is applicable to oils and fats and free fatty acids but which is less applicable to caproic acid (C6) and not applicable to butyric acid (C4). The application field is GLC, TLC and IR.

Two alternative methods not involving  $\text{BF}_3$  are given using trimethylsulfonium hydroxide and potassium hydroxide in methanol. Both methods are rapid methods for GLC analysis only.

The second method (trimethylsulfonium hydroxide method), which is for GLC analyses only, can be used for all fats and oils including milk fat and milk fat containing blends. In the case of short fatty acids (C4 to C8) the use of an internal standard is recommended.

The third method (trans-esterification method) can be used for neutral oils and fats, and can also be used for the quantitative analysis of oils and fats with short-chain fatty acids down to butyric acid (C4). For the determination of C4 and/or C6, only the internal standard method is maintained.

The principal new approach in this revision is the use of isooctane as solvent instead of hexane or pentane. This is based on references [1] and [2], which showed better results especially for the  $\text{BF}_3$  method when using isooctane.

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# Animal and vegetable fats and oils — Preparation of methyl esters of fatty acids

## 1 Scope

This International Standard specifies methods of preparing the methyl esters of fatty acids.

It includes methods for preparing fatty acid methyl esters from animal and vegetable fats and oils, fatty acids and soaps. To cover different requirements, three methylation methods are specified, as follows:

- a) boron trifluoride (BF<sub>3</sub>) method (see clause 3);
- b) trimethylsulfonium hydroxide (TMSH) method (see clause 4);
- c) trans-esterification method (see clause 5).

Methyl esters so produced are used in various analytical procedures requiring such derivatives, for example gas-liquid chromatography (GLC), thin-layer chromatography (TLC) and infrared spectrometry (IR).

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## 2 Normative references

SIST EN ISO 5509:2001

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

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

## 3 General method using boron trifluoride

**WARNING** — The method described involves the use of potentially hazardous reagents. Normal precautions shall be taken for eye protection and for protection from the dangers of corrosive chemical burns.

Boron trifluoride is poisonous. For this reason, it is not recommended that the analyst prepare the methanolic solution of boron trifluoride from methanol and boron trifluoride. (See A.1 in annex A.)

### 3.1 Principle

The glycerides are saponified with methanolic sodium hydroxide. The soaps are converted into methyl esters by reaction with a boron trifluoride/methanol complex.

For analysis of pure fatty acids and soaps, saponification with sodium hydroxide is not necessary and esters can be prepared directly by reaction with boron trifluoride.

## ISO 5509:2000(E)

## 3.2 Applicability

This method is to be preferred for most oils, fats and derivatives (fatty acids, soaps) with the exception of milk fats and of fats containing fatty acids with specific groups.

During esterification, compounds containing the following configurations may be totally or partially decomposed:

- keto, epoxy, hydroxy, hydroperoxy groupings;
- cyclopropyl and cyclopropenyl groups;
- acetylenic fatty acids.

If the fatty matter contains such compounds in only very small amounts (e.g. cottonseed oil), the method can be applied; otherwise the method described in clause 4 or 5 should be followed.

For gas chromatography, the optimum recovery of the methyl esters from the reaction mixture is obtained by using isooctane. However, only about 75 % of the methyl caproate (C6) present will be recovered.

## 3.3 Reagents

Use only reagents of recognized analytical grade.

**3.3.1 Water**, complying with grade 3 of ISO 3696.

**3.3.2 Sodium hydroxide**, methanolic solution, approximately 0,5 mol/l.

Dissolve 2 g of sodium hydroxide in 100 ml of methanol containing not more than 0,5 % (mass fraction) of water. 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.

**3.3.3 Boron trifluoride** (BF<sub>3</sub>), methanolic solution, 12 % to 15 % (mass fraction)<sup>1)</sup>. See A.1.

**3.3.4 Isooctane** (2,2,4-trimethylpentane), of chromatographic quality. See A.2.

**WARNING** — Isooctane is flammable and a fire risk. Explosive limits in air are 1,1 % to 6,0 % (volume fraction). It is toxic by ingestion and inhalation. Use a properly operating fume hood when working with this solvent.

**3.3.5 Sodium chloride**, saturated aqueous solution.

**3.3.6 Sodium sulfate**, anhydrous.

**3.3.7 Nitrogen**, having an oxygen content less than 5 mg/kg.

**3.3.8 Hexane**, of chromatographic quality, for dry methyl esters only. See A.2. Light petroleum, boiling range 40 °C to 60 °C, redistilled and residue-free, with a bromine value less than 1, may be used.

**3.3.9 Methyl red**, 1 g/l solution in 60 % (volume fraction) ethanol.

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<sup>1)</sup> 14 %, 20 % (Merck No. 8.01663) and 50 % solutions are available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.