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**Živalske in rastlinske maščobe in olja - Določevanje sestave maščobnih kislin na drugem ogljikovem položaju molekul triglicerida (ISO 6800:1997)**

Animal and vegetable fats and oils - Determination of the composition of fatty acids in the 2-position of the triglycerides molecules (ISO 6800:1997)

Tierische und pflanzliche Fette und Öle - Bestimmung der Zusammensetzung von Fettsäuren in der 2-Stellung von Triglyceridmolekülen (ISO 6800:1997)

Corps gras d'origines animale et végétale - Détermination de la composition des acides gras en position 2 dans les triglycérides (ISO 6800:1997)

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**Ta slovenski standard je istoveten z: EN ISO 6800:1997**

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**ICS:**

67.200.10	Rastlinske in živalske maščobe in olja	Animal and vegetable fats and oils
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**SIST EN ISO 6800:1998****en**

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EUROPEAN STANDARD  
NORME EUROPÉENNE  
EUROPÄISCHE NORM

EN ISO 6800

December 1997

ICS 67.200.00

Descriptors: see ISO document

English version

Animal and vegetable fats and oils - Determination of the  
composition of fatty acids in the 2-position of the triglycerides  
molecules (ISO 6800:1997)

Corps gras d'origines animale et végétale - Détermination  
de la composition des acides gras en position 2 dans les  
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Tierische und pflanzliche Fette und Öle - Bestimmung der  
Zusammensetzung von Fettsäuren in der 2-Stellung von  
Triglyceridmolekülen (ISO 6800:1997)

This European Standard was approved by CEN on 9 January 1998.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

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.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.



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

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

Page 2  
EN ISO 6800:1997

## Foreword

The text of the International Standard ISO 6800:1997 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 June 1998, and conflicting national standards shall be withdrawn at the latest by June 1998.

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.

## Endorsement notice

The text of the International Standard ISO 6800:1997 was approved by CEN as a European Standard without any modification.

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

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**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
ISO 5508	1990	Animal and vegetable fats and oils - Analysis by gas chromatography of methyl esters of fatty acids	EN ISO 5508	1995

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# INTERNATIONAL STANDARD

**ISO  
6800**

Second edition  
1997-12-15

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## **Animal and vegetable fats and oils — Determination of the composition of fatty acids in the 2-position of the triglyceride molecules**

*Corps gras d'origines animale et végétale — Détermination de la  
composition des acides gras en position 2 dans les triglycérides*

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Reference number  
ISO 6800:1997(E)

## ISO 6800:1997(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.

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.

International Standard ISO 6800 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 6800:1985), which has been technically revised.

Annexes A to C of this International Standard are for information only.

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# Animal and vegetable fats and oils — Determination of the composition of fatty acids in the 2-position of the triglyceride molecules

## 1 Scope

This International Standard specifies a method for the determination of the composition of fatty acids which are esterified in the 2-position ( $\beta$  or internal position) of the triglyceride molecules in animal and vegetable fats and oils.

Because of the nature of pancreatic lipase action, the method is applicable only to fats and oils with a melting point below 45 °C.

The method is not unreservedly applicable to all fats and oils, particularly those containing substantial amounts of

- fatty acids with 12 or fewer carbon atoms (e.g. copra oil, palm kernel oil, butyric butter fats);
- fatty acids with 20 and more carbon atoms and of a high degree of unsaturation (more than four double bonds) (e.g. fish oil and marine animal oil);
- fatty acids which have secondary groups containing oxygen.

NOTE — Fatty acids with double bonds in the ( $n$ -16) to ( $n$ -11) position (e.g. petroselinic acid) are converted only very slowly by pancreatic lipase. This may lead to wrong results.

## 2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 660:1996, *Animal and vegetable fats and oils – Determination of acid value and of acidity*.

ISO 661:1989, *Animal and vegetable fats and oils – Preparation of test sample*.

ISO 3696:1987, *Water for analytical laboratory use – Specification and test methods*.

ISO 5508:1990, *Animal and vegetable fats and oils – Analysis by gas chromatography of methyl esters of fatty acids*.

ISO 5509:—<sup>1)</sup> *Animal and vegetable fats and oils – Preparation of methyl esters of fatty acids.*

### 3 Principle

After neutralization, where necessary, of any free fatty acids, purification of the test portion by column chromatography. Partial enzymatic hydrolysis of the glycerides to yield 2-monoglycerides. Separation of the monoglycerides by thin-layer chromatography (TLC) and determination of their fatty acid composition by gas chromatography.

### 4 Reagents

Use only reagents of recognized analytical grade and water in accordance with grade 2 of ISO 3696.

#### 4.1 Reagents for the purification of the test portion

**4.1.1 2-Propanol**, or **ethanol**, 95 % (V/V).

**4.1.2 Hexane** (if available) or **light petroleum** (boiling range 30 °C to 60 °C).

**4.1.3 2-Propanol**, 50 % (V/V), or **ethanol**, 50 % (V/V).

**4.1.4 Sodium hydroxide**, 0,5 mol/l solution.

**4.1.5 Phenolphthalein solution**, 1 g per 100 ml of ethanol, 95 % (V/V).

**4.1.6 Activated neutral alumina**, for chromatography, Brockmann activity I, recently activated for 2 h at 260 °C and kept in a desiccator.

**4.1.7 Nitrogen.**

#### 4.2 Reagents for hydrolysis of the triglycerides

**4.2.1 Diethyl ether**, free from peroxides.

**4.2.2 Hydrochloric acid**, 6 mol/l solution.

**4.2.3 Sodium cholate**, 1 g/l solution of enzymatic quality.

**4.2.4 Calcium chloride**, 220 g/l solution.

**4.2.5 Buffer solution**, 2-amino-2-(hydroxymethyl)propan-1,3-diol<sup>2)</sup>, 1 mol/l, adjusted to pH 8 with hydrochloric acid (4.2.2) using a pH-meter.

Store this solution at between 0 °C and 4 °C and use within 14 days.

1) To be published. (Revision of ISO 5509:1978)

2) Alternative names are: tris(hydroxymethyl)methylamine; tris(hydroxymethyl)aminomethane.

#### 4.2.6 **Pancreatic lipase**, with an activity of between 8 and 20 units/mg.

Store dry in a refrigerator. Before use, bring a portion of the powder to ambient temperature.

NOTE — Lipase of suitable activity is available commercially. If preferred, the lipase may be prepared and assayed in accordance with the procedure described in annex A.

### 4.3 **Reagents for the isolation of the 2-monoglycerides**

#### 4.3.1 **Ethanol**, 95 % (V/V).

#### 4.3.2 **Hexane** (if available) or **light petroleum**, boiling range 30 °C to 60 °C.

#### 4.3.3 **Acetone**.

#### 4.3.4 **Silica powder**, with binder, for thin-layer chromatography.

#### 4.3.5 **Developing solvent**, prepared as follows:

hexane (if available) or light petroleum:	70 ml
diethyl ether:	30 ml
formic acid, 98 % (V/V) min.:	1 ml

#### 4.3.6 **2',7'-Dichlorofluorescein**, indicator solution, 2 g/l in ethanol, rendered slightly alkaline by addition of a drop of 1 mol/l sodium hydroxide per 100 ml of the solution.

### 4.4 **Reagents for the analysis of the 2-monoglycerides by gas chromatography**

See ISO 5508 and ISO 5509.

## 5 **Apparatus**

Usual laboratory equipment and, in particular, the following.

### 5.1 **Apparatus for the purification of the test portion**

#### 5.1.1 **Water bath**, thermostatically controlled, and capable of being maintained at 30 °C to 40 °C.

#### 5.1.2 **Glass column**, for chromatography, 13 mm internal diameter and 400 mm in length, equipped with a sintered glass plate and a tap.

#### 5.1.3 **Rotary evaporator**, with 250 ml flask.

#### 5.1.4 **Tubing**, for bubbling nitrogen.

#### 5.1.5 **Separating funnel**, of 500 ml capacity.

#### 5.1.6 **Round-bottom flask**, of 100 ml capacity.