SLOVENSKI STANDARD
SIST EN ISO 9936:2006

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Rastlinske in ģivalske maġļ obe in olja ï Doloļ evanje tokoferola in tokotrienola s tekoļ insko kromatografijo visoke loļ ljivosti (ISO 9936:2006)

Animal and vegetable fats and oils - Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography (ISO 9936:2006)

Tierische und pflanzliche Fette und Öle - Bestimmung des Tocopherol- und Tocotrienol-Gehaltes mit Hochleistungsflüssigchromatographie (ISO 9936:2006)

Corps gras d'origines animale et végétale - Détermination des teneurs en tocophérols et en tocotriénols par chromatographie en phase liquide a haute performance (ISO 9936:2006)

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Animal and vegetable fats and oils - Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography (ISO 9936:2006)

Corps gras d'origines animale et végétale - Détermination des teneurs en tocophérols et en tocotriénols par chromatographie en phase liquide à haute performance (ISO 9936:2006)

Tierische und pflanzliche Fette und Öle - Bestimmung des Tocopherol- und Tocotrienol-Gehaltes mit Hochleistungsflüssigchromatographie (ISO 9936:2006)

This European Standard was approved by CEN on 13 April 2006.

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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
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Foreword

This document (EN ISO 9936:2006) 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of an identical text or by endorsement, at the latest by October 2006, and conflicting national standards shall be withdrawn at the latest by October 2006.

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

Endorsement notice

The text of ISO 9936:2006 has been approved by CEN as EN ISO 9936:2006 without any modifications.

INTERNATIONAL STANDARD

ISO 9936

Second edition
2006-04-15

Animal and vegetable fats and oils — Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography

Corps gras d'origines animale et végétale — Détermination des teneurs en tocophérols et en tocotriénols par chromatographie en phase liquide à haute performance

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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 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 9936 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 9936:1997), which has been technically revised.

Animal and vegetable fats and oils — Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography

1 Scope

This International Standard specifies a method for the determination of the contents of free α-, β-, γ-, and δ-tocopherols and tocotrienols (referred to jointly as tocols) in animal and vegetable fats and oils (referred to hereinafter as fats) by high-performance liquid chromatography (HPLC).

For products containing tocopherol or tocotrienol esters, it is necessary to carry out a preliminary saponification.

NOTE A suitable method involving a cold saponification procedure is described in Annex B for information only.

2 Normative references

The following referenced documents are indispensable for the application 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*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
tocol content
mass fraction of the individual tocols, determined using the method specified in this International Standard

NOTE The content is expressed in milligrams per kilogram as a whole number.

4 Principle

A test portion is dissolved in *n*-heptane and the individual tocols are separated by high-performance liquid chromatography. The content of each tocol is calculated using calibration factors determined from calibration solutions.

5 Reagents

Use only reagents of HPLC grade or equivalent.

5.1 α-, β-, γ- and δ-tocopherol and tocotrienol standards.

If tocopherol standards are not available, a blend of wheat germ and soya bean oil may be used to identify α-, β-, γ- and δ-tocopherols.

If tocotrienol standards are not available, palm oil may be used to identify α- and γ-tocotrienols. The chromatograms obtained can be used to assist peak identification in test sample chromatograms, in which case the calibration factors for the corresponding tocopherols should be used.

NOTE α-, β-, γ- and δ-tocopherol and tocotrienol standards can be obtained from Merck [1)]; α-tocopherol can be obtained from various suppliers. It has been reported that the purity of some commercially available tocopherol standards may vary between 85 % and 100 %. Thus, it is important to determine the concentration of prepared calibration solutions by UV spectrometry (see 9.1.1).

5.2 Tetrahydrofuran, filtered through an HPLC nylon filter (0,45 µm).

5.3 *n*-Heptane, filtered through an HPLC nylon filter (0,45 µm).

5.4 HPLC mobile phase: any suitable mixture of solvents that has been proved to reach a chromatographic resolution of peaks as good as the one presented in Table 2 (relative retention time of tocopherols and tocotrienols) and in Annex A (chromatograms of a mixture of vegetable oils), should be used (see Table C.3).

The preparation of a suitable mobile phase, 3,85 % (volume fraction) tetrahydrofuran solution in *n*-heptane, is as follows. Using a 1 000 ml graduated cylinder (6.5), introduce 1 000 ml of *n*-heptane (5.3) in a 2 litre bottle. Add twice 20 ml of tetrahydrofuran (5.2) using a 20 ml volumetric pipette (6.6). Homogenize the mobile phase by means of an ultrasonic bath (6.8) for 15 min.

5.5 Methanol.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 HPLC system, consisting of a high-pressure pump, a sample injection device, column thermostat adjusted to 25 °C (optional), a fluorescence detector with the excitation wavelength set at 295 nm and emission wavelength at 330 nm, and a recording integrator.

An ultraviolet (UV) detector may be used if a fluorescence detector is not available but it is not recommended. However, if a UV detector is used, the wavelength should be set at 292 nm.

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1) Merck Tocopherol set 613424 is available from Calbiochem (www.calbiochem.com). It contains one 50 mg vial each of DL-α-tocopherol, D-β-tocopherol, D-γ-tocopherol, and D-δ-tocopherol with a purity of 95 % by HPLC (for each component). Merck Tocotrienol set 613432 is available from Calbiochem also. It contains one 50 mg vial each of α-tocotrienol, β-tocotrienol, γ-tocotrienol, and δ-tocotrienol with a purity of 95 % by HPLC (75 % for γ-tocotrienol).

This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

6.2 HPLC analytical column, two types are possible:

— 250 mm × 4 mm, packed with microparticulate **diol** having a mean particle size of about 5 µm, or

— 250 mm × 4,6 mm, packed with microparticulate **silica** having a mean particle size of about 5 µm.

NOTE 1 Suitable diol silica column packing material available commercially is 5 µm LiChrospher 100 Diol; suitable silica column packing materials available commercially are 5 µm LiChrosob SI 60 and Kromasil 100 [2]. When β-tocotrienol is expected in the sample, the diol silica column is preferred as γ-tocopherol and β-tocotrienol are co-eluted when using the silica column.

NOTE 2 The length and the diameter of the column can be varied according to the HPLC technique used.

6.3 UV spectrometer, capable of absolute measurement of absorbance at precisely defined wavelengths, with a 10-mm path length cell.

6.4 Rotary evaporator.

6.5 Graduated cylinder, of 1 000 ml capacity.

6.6 Volumetric pipettes, of 5 ml, 10 ml and 20 ml capacities.

6.7 Volumetric flasks, 50 ml and 25 ml capacities.

6.8 Ultrasonic bath.

7 Sampling

A representative sample should be sent to the laboratory. It is important that the sample has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 5555.

8 Preparation of test sample

In the case of liquid laboratory samples, prepare the test sample by homogenization as described in ISO 661, except that filtration should be avoided.

In the case of solid samples, transfer a representative portion (i.e. not less than 10 % by mass of the laboratory sample) to a glass beaker and carefully homogenize by melting, with gentle mixing, in a water bath at a temperature not exceeding 40 °C.

Preparation of the test samples should be carried out, as far as is practicable, in subdued light and in all cases out of direct sunlight.

2) These types of columns are examples of suitable products which are available commercially.

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