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

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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 de la teneur en tocophérol et en tocotriénol par chromatographie en phase liquide à haute performance

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

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Foreword

This document (prEN ISO 9936:2004) has been prepared by Technical Committee ISO/TC 34 " Animal and vegetable fats and oils" 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 document is currently submitted to the parallel Enquiry.

Endorsement notice

The text of ISO/DIS 9936:2004 has been approved by CEN as prEN ISO 9936:2004 without any modifications.

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Corps gras d'origines animale et végétale — Détermination de la teneur en tocophérol et en tocotriénol par chromatographie en phase liquide à haute performance

[Revision of first edition (ISO 9936:1997)]

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Conformément aux dispositions de la Résolution du Conseil 15/1993, ce document est distribué en version anglaise seulement.

To expedite distribution, this document is circulated as received from the committee secretariat. ISO Central Secretariat work of editing and text composition will be undertaken at publication stage.

Pour accélérer la distribution, le présent document est distribué tel qu'il est parvenu du secrétariat du comité. Le travail de rédaction et de composition de texte sera effectué au Secrétariat central de l'ISO au stade de publication.

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Foreword

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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 prepare the unsaponifiable matter.

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

2 Normative reference

The following referenced document is 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.*

ISO 5555, *Animal and vegetable fats and oils — Sampling*.

3 Definition

For the purpose of this International Standard, the following definition applies.

3.1
tocols contents
Mass fractions 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

All reagents shall be 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 can be used to identify α-, β-, γ- and δ-tocopherols.

If tocotrienol standards are not available, palm oil can 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 tocotrienols 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, filtrated on a HPLC nylon filter (0,45 µm).

5.3 *n*-Heptane, filtrated on a HPLC nylon filter (0,45 µm).

5.4 HPLC mobile phase: any suitable mixture of solvents, 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 Annex D).

The preparation of a suitable mobile phase, 3,85 % (*V/V*) tetrahydrofuran solution in *n*-heptane, is given here: using a 1000 ml graduated cylinder (6.5), introduce 1000 ml of n-heptane (5.3) in a 2 liter bottle. Add twice 20 ml of tetrahydrofuran (5.2) using a 20 ml volumetric pipette (6.6). Homogenize the mobile phase and put it 15 minutes in an ultrasonic bath (6.8).

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.

NOTE 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.

6.2 HPLC analytical column, 2 types possible:

250 mm x 4 mm, packed with micro particulate **diol silica** having a mean particle size of about 5 µm, or 250 mm x 4,6 mm, packed with micro particulate **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

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[1] Merck Tocopherol set 613424 is available from Calbiochem (www.calbiochem.com). 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. 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).

[2] These types of columns are examples of suitable products which 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.