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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 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.

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 documents 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).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This third edition cancels and replaces the second edition (ISO 9936:2006), which has been technically revised. It also incorporates the Amendment ISO 9936:2006/Amd.1:2011 and the Technical Corrigendum ISO 9936:2006/Cor.1:2008. A non-applicability statement for milk and milk products has been added to the Scope.

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.

Milk and milk products (or fat coming from milk and milk products) are excluded from the Scope of this International Standard.

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

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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 1 to entry: 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 (HPLC). 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. Tocotrienol standards are available from Sigma Aldrich[2)]. 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 proven 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).

A good separation of γ-tocopherol and β-tocotrienol can be achieved by using a mixture of 5 % volume fraction *t*-butyl methyl ether + 95 % volume fraction *n*-heptane and a diol-column.

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.

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.

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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 document and does not constitute an endorsement by ISO of the products named. Equivalent products may be used if they can be shown to lead to the same results.

2) Tocotrienols are available from Sigma Aldrich (www.sigmaaldrich.com) and from Chromadex (www.chromadex.com) with purities between 65 % and 98 %. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the products named. Equivalent products may be used if they can be shown to lead to the same results.

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[3]. 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.

NOTE 3 Both types of columns have been used for the evaluation of the precision data (Annex C).

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.

9 Procedure

IMPORTANT — In general, the oxidation of tocols during the analysis may lead to low results. The rate of oxidation is increased in the presence of alkalis, or under the influence of heat or light, and measures should be taken to guard against these influences.

3) These types of columns are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.