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SLOVENSKI STANDARD
SIST ISO 9936:1998

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Animal and vegetable fats and oils -- Determination of tocopherols and tocotrienols contents -- Method using high-performance liquid chromatography

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

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ICS:

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INTERNATIONAL STANDARD

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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.

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.

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International Standard ISO 9936 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

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

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Animal and vegetable fats and oils — Determination of tocopherols and tocotrienols contents — Method using 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.

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

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2 Normative reference

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

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

3 Definition

For the purposes 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 They are expressed in micrograms per gram.

4 Principle

A test portion is dissolved 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.

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5 Reagents

All reagents shall be of HPLC grade or equivalent.

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

5.1.1 If tocopherol standards are not available, a blend of wheat germ and soya bean oil can be used to obtain chromatograms which contain α-, β-, γ- and δ-tocopherols.

5.1.2 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 β-, γ- and δ-tocopherol standards can be obtained from Merck; 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.

5.2 Methanol.

5.3 Dichloromethane.

5.4 *n*-Hexane.

5.5 Propan-2-ol.

5.6 HPLC mobile phase, propan-2-ol, 0,5 % (*V*/*V*) solution in *n*-hexane.

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6 Apparatus

All glassware shall be of low actinic activity. No apparatus shall give an alkaline reaction. This requirement is not applicable when solutions are protected by pyrogallol.

Usual laboratory apparatus and, in particular, the following.

6.1 HPLC system, consisting of a high-pressure pump, a sample injection device, a fluorescence detector with the excitation wavelength set at 290 nm and emission wavelength at 330 nm, and a chart recorder or 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, 250 mm x 4 mm, packed with microparticulate silica having a mean particle size of about 5 µm.

NOTE 1 Suitable silica column packing materials available commercially are 5 µm LiChrosob SI 60 and Spherisorb S5W [1)].

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

NOTE 3 Depending on the silica HPLC column status (prehistory, dry or deactivation by traces of water, etc.) a triacylglycerol peak could overlap the *α*-tocopherol peak.

If this happens, different results may be obtained from a fluorescence detector (possibility of quenching) and a UV detector (peak disturbance). This will be most problematic if a calibration is carried out with a calibrant that does not contain fat for the analysis of fat-containing samples.

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

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6.3 UV spectrometer, capable of absolute measurement of absorbance at precisely defined wavelengths.

6.4 Rotary evaporator.

7 Sampling

It is important that the laboratory receive a sample which is truly representative and 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 [2].

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 any case out of direct sunlight.

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9 Procedure

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NOTE In general, the oxidation of tocols during the analysis may lead to low results. In the presence of oxygen they are oxidized more quickly in the presence of alkalis, or under the influence of heat or light, and measures should be taken to guard against these influences.

9.1 Preparation of calibration solutions

9.1.1 Stock calibration solutions

Prepare a stock solution of each tocol by weighing 10 mg ± 1 mg of the standard (5.1) into a 100 ml one-mark volumetric flask and diluting to the mark with hexane (5.4).

Pipette 10 ml of this solution into an amber glass round-bottomed flask and remove all hexane on a rotary evaporator (6.4) under vacuum at a temperature not greater than 40 °C. Restore atmospheric pressure with nitrogen and remove the flask from the evaporator as soon as all the solvent has been removed. Pipette into the flask 10 ml of methanol (5.2) and swirl to dissolve the residue. Measure the absorbance of this solution at the appropriate wavelength using the UV spectrometer (6.3). Calculate the concentration (in micrograms per millilitre) by dividing the absorbance value by the appropriate factor given in table 1.