SLOVENSKI STANDARD
SIST EN ISO 15788-2:2005

01-junij-2005

Živalske in rastlinske maščobe in olja - Ugotavljanje stigmastadienov v rastlinskih oljih - 2. del: Metoda s tekočinsko kromatografijo visoke ločljivosti (HPLC) (ISO 15788-2:2003)

Animal and vegetable fats and oils - Determination of stigmastadienes in vegetable oils - Part 2: Method using high-performance liquid chromatography (HPLC) (ISO 15788-2:2003)

Tierische und pflanzliche Fette und Öle - Bestimmung der Stigmastadiene in Pflanzenölen - Teil 2: Verfahren mit Hochleistungsflüssigchromatographie (HPLC) (ISO 15788-2:2003)

Corps gras d'origines animale et végétale - Dosage des stigmastadienes dans les huiles végétales - Partie 2: Méthode par chromatographie liquide a haute performance (CLHP) (ISO 15788-2:2003)

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ICS:

67.200.10	Rastlinske in živalske maščobe in olja	Animal and vegetable fats and oils

SIST EN ISO 15788-2:2005 **en**

EUROPEAN STANDARD

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EN ISO 15788-2

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ICS 67.200.10

English version

Animal and vegetable fats and oils - Determination of stigmastadienes in vegetable oils - Part 2: Method using high-performance liquid chromatography (HPLC) (ISO 15788-2:2003)

Corps gras d'origines animale et végétale - Dosage des stigmastadiènes dans les huiles végétales - Partie 2: Méthode par chromatographie liquide à haute performance (CLHP) (ISO 15788-2:2003)

Tierische und pflanzliche Fette und Öle - Bestimmung der Stigmastadiene in Pflanzenölen - Teil 2: Verfahren mit Hochleistungsflüssigchromatographie (HPLC) (ISO 15788-2:2003)

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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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EUROPÄISCHES KOMITEE FÜR NORMUNG

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Ref. No. EN ISO 15788-2:2004: E

Foreword

The text of ISO 15788-2:2003 has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 15788-2:2004 by 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 May 2005, and conflicting national standards shall be withdrawn at the latest by May 2005.

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, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

Endorsement notice

The text of ISO 15788-2:2003 has been approved by CEN as EN ISO 15788-2:2004 without any modifications.

INTERNATIONAL STANDARD

ISO 15788-2

First edition
2003-02-15

Animal and vegetable fats and oils — Determination of stigmastadienes in vegetable oils —

Part 2: Method using high-performance liquid chromatography (HPLC)

Corps gras d'origines animale et végétale — Dosage des stigmastadiènes dans les huiles végétales —

Partie 2: Méthode par chromatographie liquide à haute performance (CLHP)

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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 15788-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

ISO 15788 consists of the following parts, under the general title *Animal and vegetable fats and oils — Determination of stigmastadienes in vegetable oils*:

— *Part 1: Method using capillary-column gas chromatography (Reference method)*

— *Part 2: Method using high-performance liquid chromatography (HPLC)*

Animal and vegetable fats and oils — Determination of stigmastadienes in vegetable oils —

Part 2: Method using high-performance liquid chromatography (HPLC)

1 Scope

This part of ISO 15788 specifies a method for the determination of steradienes, especially stigmastadienes. Steradienes are formed by dehydration of sterols during bleaching and also partially during steam washing and deodorization. The method is also suitable as a screening method to detect the presence of refined vegetable oils in virgin oils such as virgin olive oil.

NOTE ISO 15788-1 is the reference method for the determination of stigmastadienes in vegetable oils whilst this part of ISO 15788 can be used as a rapid screening method. In view of the precision of this method (see Annex A), samples of virgin olive oils close to the limit adopted by international regulations (IOOC, EC) can be verified by the GLC method given in ISO 15788-1.

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 3696:1987, *Water for analytical use — Specification and test method*

ISO 12228, *Animal and vegetable fats and oils — Determination of individual and total sterols content — Gas chromatographic method*

3 Terms and definitions

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

3.1
stigmastadienes content
that part of the stigmastadienes separated by liquid chromatography under the conditions specified in this International Standard

NOTE It is expressed in milligrams per kilogram.

3.2
steradienes content
that part of all the steradienes separated by liquid chromatography under the conditions specified in this International Standard

NOTE It is expressed in milligrams per kilogram.

4 Principle

Steradienes are separated from the major part of other lipids as non-polar fat compounds, using petroleum ether on a silica gel column. The petroleum ether eluate is concentrated then analysed by RP-18-HPLC and UV-detection at 235 nm. They are quantified, depending on the kind of sample, using an internal or external standard.

5 Reagents

WARNING — Attention is drawn to the regulations which prescribe the handling of dangerous matter. Technical, organizational and personal safety measurements shall be followed.

Use only reagents of recognized analytical grade, unless otherwise stated.

5.1 Water, of at least grade 1 according to ISO 3696:1987.

5.2 Silica gel 60 for column chromatography, particle size 0,063 mm to 0,200 mm, or 0,063 mm to 0,100 mm[1)], with a water content of 2 g per 100 g.

Dry the silica gel in a porcelain dish for 12 h at 160 °C in a drying oven and cool to room temperature in a desiccator. For adjusting the silica gel to a water content of 2 g per 100 g, weigh (to the nearest 1 g) 98 g of the dried silica gel in a conical flask with ground glass stopper and add 2 g of water (weighed to the nearest 0,01 g). Shake vigorously for 1 min and allow the silica gel to stand overnight in an airtight vessel.

5.3 Petroleum ether, boiling range 40 °C to 60 °C.

5.4 Acetonitrile, chromatography grade.

5.5 *tert*-Butyl methyl ether, chromatography grade.

5.6 Isooctane.

5.7 Δ3,5-Cholestadiene[2)], with a known purity of at least 95 g per 100 g.

Check the purity of the cholestadiene standard by gas chromatography using 5α-cholestane as internal standard. For this test, see the conditions for the gas chromatographic method for the determination of sterols specified in ISO 12228. The response factor of the flame ionization detector should be 1,0. Take into consideration the obtained concentration during the determination of the steradienes.

5.8 Stock solutions and standard solutions

5.8.1 Δ3,5-Cholestadiene stock solution, of concentration 1 mg/ml.

Weigh, to the nearest 0,1 mg, 50,0 mg of Δ3,5-cholestadiene into a 50 ml measuring flask. Dissolve and dilute to the mark with *tert*-butyl methyl ether (5.5).

5.8.2 Δ3,5-Cholestadiene standard solution for HPLC

1) For example, No. 7734 or 15101 from E. Merck, 64271 Darmstadt, Germany.

2) For example, No. C6012 from Sigma Chemie GmbH, Grünwalder Weg 30, 82041 Deisenhofen, Germany.

This information is given for the convenience of users of this part of ISO 15788 and does not constitute an endorsement by ISO of these products.