SLOVENSKI STANDARD
SIST EN ISO 15753:2007

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Rastlinske in ģivalske maģļ obe in olja - Doloļ evanje policikliļ nih aromatskih ogljikovodikov (ISO 15753:2006)

Animal and vegetable fats and oils - Determination of polycyclic aromatic hydrocarbons (ISO 15753:2006)

Tierische und pflanzliche Fette und Öle - Bestimmung von polycyclischen aromatischen Kohlenwasserstoffen (ISO 15753:2006)

Corps gras d'origines animale et végétale - Détermination des hydrocarbures aromatiques polycycliques (ISO 15753:2006)

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Corps gras d'origines animale et végétale - Détermination des hydrocarbures aromatiques polycycliques (ISO 15753:2006)

Tierische und pflanzliche Fette und Öle - Bestimmung von polycyclischen aromatischen Kohlenwasserstoffen (ISO 15753:2006)

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Foreword

This document (EN ISO 15753:2006) 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of an identical text or by endorsement, at the latest by March 2007, and conflicting national standards shall be withdrawn at the latest by March 2007.

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Endorsement notice

The text of ISO 15753:2006 has been approved by CEN as EN ISO 15753:2006 without any modifications.

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Corps gras d'origines animale et végétale — Détermination des hydrocarbures aromatiques polycycliques

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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 15753 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

Animal and vegetable fats and oils — Determination of polycyclic aromatic hydrocarbons

1 Scope

This International Standard describes two methods for the determination of 15 polycyclic aromatic hydrocarbons (PAHs) in animal and vegetable fats and oils:

— a general method, and

— a method specific for coconut oil and vegetable oils with short-chain fatty acids.

These methods are not quantitative for the very volatile compounds such as naphthalene, acenaphthene and fluorene. Due to interferences provided by the matrix itself, palm oil and olive pomace oil cannot be analysed using this method.

The quantification limit is 0,2 µg/kg for almost all compounds analysed, except for fluoranthene and benzo(*g*,*h*,*i*)perylene where the quantification limit is 0,3 µg/kg, and indeno(1,2,3-*c*,*d*)pyrene where the quantification limit is 1 µg/kg.

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
polycyclic aromatic hydrocarbon
PAH
compound that contains two or more condensed (fused) aromatic hydrocarbon rings and the content of which can be determined according to the method specified in this International Standard

NOTE 1 The content is given in micrograms per kilogram.

NOTE 2 In general PAHs are divided into light PAHs with two to four aromatic rings, and heavy PAHs with five or more aromatic rings.

EXAMPLES

Light PAHs include:
naphthalene (CAS RN [91-20-3]), acenaphthene (CAS RN [83-32-9]), acenaphthylene (CAS RN [208-96-8]), fluorene (CAS RN [86-73-7]), anthracene (CAS RN [120-12-7]), phenanthrene (CAS RN [85-01-8]), fluoranthene (CAS RN [206-44-0]), chrysene (CAS RN [218-01-9]), benz(*a*)anthracene (CAS RN [56-55-3]), pyrene (CAS RN [129-00-0]).

Heavy PAHs include:
benzo(*a*)pyrene (CAS RN [50-32-8]), benzo(*b*)fluoranthene (CAS RN [205-99-2]), benzo(*k*)fluoranthene (CAS RN [207-08-9]), benzo(*g*,*h*,*i*)perylene (CAS RN [191-24-2]), dibenz(*a*,*h*)anthracene (CAS RN [53-70-3]), indeno(1,2,3-*c*,*d*)pyrene (CAS RN [193-39-5]).

4 Principle

The polycyclic aromatic hydrocarbons are extracted with an acetonitrile/acetone mixture followed by purification on C18 reversed-phase and then Florisil bonded-phase cartridges. Determination of the content of the individual polycyclic aromatic hydrocarbons after separation is achieved by means of high-pressure liquid chromatography (HPLC) and by measuring the fluorescence at various excitation and emission wavelengths.

5 Reagents and materials

WARNING — Attention is drawn to the regulations governing the handling of dangerous matter. Technical, organizational and personal safety measures must be followed.

Use only reagents of recognized analytical grade unless otherwise stated.

Check the quality of solvents before use by concentrating the solvent about 1 000 times by evaporation and analysing the concentrate by HPLC (300 ml to 300 µl). The chromatogram shall be free from peaks in the elution area of PAHs.

5.1 Methanol, 'ultra resi-analysed' grade[1)].

5.2 Hexane, HPLC grade[1)].

5.3 Acetonitrile, HPLC grade[1)].

5.4 Acetone, HPLC grade[1)].

5.5 Dichloromethane, HPLC grade[1)].

5.6 Toluene, HPLC grade[1)].

5.7 Water, HPLC grade[1)].

5.8 Tetrahydrofuran, HPLC grade[1)].

5.9 Solvent mixture 1: acetonitrile/acetone (volume fraction 60 % / 40 %).

Quantity used per sample: 41 ml for general method, 36 ml for method specific for coconut oil.

5.10 Solvent mixture 2: acetonitrile/acetone (volume fraction 80 % / 20 %).

Quantity used per sample: 2 × 11 ml for method specific for coconut oil.

5.11 Solvent mixture 3: hexane/dichloromethane (volume fraction 75 % / 25 %).

Quantity used per sample: 7 ml for general method, 2 × 7 ml for method specific for coconut oil.

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1) These can be obtained from, for example, Baker.

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5.12 Mixture of tetrahydrofuran/methanol (volume fraction 50 % / 50 %).

5.13 Standard solution with 16 certified EPA Priority PAHs in toluene[2], at a concentration of 100 µg/ml (100 mg/l): naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, dibenz(*a*,*h*)anthracene, benzo(*g*,*h*,*i*)perylene, indeno(1,2,3-*c*,*d*)pyrene. To be stored at −20 °C.

Before use, allow the solution to warm up to ambient temperature for at least 1 h.

NOTE Acenaphthylene is not fluorescent and, thus, it cannot be determined by these methods.

5.14 Stock standard solution, 200 ng/ml (200 µg/l).

Add 100 µl of standard solution (5.13) with a 250 µl syringe (6.11) to a 50 ml volumetric flask (6.20) and dilute to the mark with acetonitrile.

5.15 Working standard solution, 50 ng/ml (50 µg/l).

Add 250 µl of stock standard solution (5.14) with a 250 µl syringe (6.11) to 750 µl of THF/methanol mixture (5.12) or acetonitrile (5.3).

5.16 C18 bonded-phase cartridges[3], 2 g phase, 12 ml capacity.

5.17 Florisil bonded-phase cartridges[3], 500 mg phase, 3 ml capacity.

5.18 Stream of nitrogen, pressure regulated at 34,5 kPa (5 psi, about 1,5 l/min).

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

The use of disposable glass tubes is acceptable. The general use of glass is necessary as plastics can contain PAHs.

6.1 Centrifuge, capable of attaining at least 4 000 min^{-1}, suitable for 100 ml and 10 ml tubes.

6.2 HPLC system with binary gradient elution, with solvent reservoir of 1 l capacity, a mobile phase liner filter, pump, autosampler, column temperature regulation set at 25 °C, fluorescence detector programmable over time for various excitation and emission wavelengths, and computer-assisted acquisition and data treatment.

6.3 C18 reversed-phase column[4], 250 mm in length, 4,6 mm internal diameter, 5 µm particles, suitable for PAH analysis.

6.4 Vortex mixer.

6.5 Automatic evaporator[5], for 10 ml tube (optional), or water bath (6.6).

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2) This can be obtained from, for example, Promochem.

3) This can be obtained from, for example, Varian.

This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products. Equivalent products may be used if they can be shown to lead to the same results.

4) This can be obtained from, for example, Vydac, ref. 201TP54.

5) This can be obtained from, for example, Zymark, Zymark TurboVap LV evaporator.

Recommended operating conditions:

— temperature of water bath 35 °C;

— nitrogen pressure 34,5 kPa.

6.6 Water bath, regulated at 35 °C.

6.7 Balance, with readability of 0,1 mg.

6.8 Centrifuge tubes, of 100 ml capacity (one per sample).

6.9 Conical centrifuge tubes, of 11 ml capacity (three per sample), with PTFE septa and closed top screw caps (one per sample).

6.10 Graduated cylinders.

6.11 Microsyringe, 250 µl.

6.12 Syringe, 1 000 µl.

6.13 Graduated pipette, 5 ml.

6.14 Syringe, 5 ml, equipped with an adapter cap for SPE cartridges.

6.15 Vials for autosampler.

6.16 Microvials, of 250 µl capacity, adapted for HPLC system.

6.17 Ultrasonic bath, with water temperature not higher than 40 °C.

6.18 Pasteur pipettes, with cotton wool in the top part to prevent contamination.

6.19 Device composed of stand and pincers[6], to hold SPE cartridges or, if available, an automatic SPE work station.

NOTE Depending on the SPE sample processing station used, the proposed extraction methods may require slight adaptations (times, pressure, volumes).

6.20 Volumetric flask, of capacity 50 ml.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have 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.

6) This can be obtained from, for example, Zymark, Zymark Rapid Trace.

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