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

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

Reference number
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Foreword

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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 second edition cancels and replaces the first edition (ISO 15753:2006), of which it constitutes a minor revision. It also incorporates Amendment ISO 15753:2006/Amd.1:2011. A non-applicability statement for milk and milk products has been added to the Scope.

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;

— a specific method 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 1,0 µg/kg.

NOTE The results for olive pomace oil in Annex B show that this method is not applicable to this type of oil. The precision data determined are very poor.

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

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
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 to entry: The content is given in micrograms per kilogram.

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

EXAMPLE 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 should 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 specific method 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.

1) These can be obtained from, for example, Baker. The information given in the footnotes is for the convenience of users of this document 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.

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.

NOTE 1 This is stored at -20 °C.

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

NOTE 2 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.

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

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

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