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Livsmedel – Vegetabiliska oljor och livsmedel baserade på vegetabiliska oljor – Bestämning av mättade kolväten från mineralolja (MOSH) och aromatiska kolväten från mineralolja (MOAH) med online HPLC-GC-FID-analys

Foodstuffs – Vegetable oils and foodstuff on basis of vegetable oils – Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis

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The European Standard EN 16995:2017 has the status of a Swedish Standard. This document contains the official version of EN 16995:2017.

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Denna standard är framtagen av kommittén för Livsmedel och foder, SIS/TK 435.

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EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 16995

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English Version

Foodstuffs - Vegetable oils and foodstuff on basis of vegetable oils - Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis

Produits alimentaires - Huiles végétales et produits alimentaires à base d'huiles végétales - Dosage des hydrocarbures saturés d'huile minérale (MOSH) et des hydrocarbures aromatiques d'huile minérale (MOAH) par analyse par CLHP-CG-FID en ligne

Lebensmittel - Pflanzliche Öle und Lebensmittel auf Basis pflanzlicher Öle - Bestimmung von gesättigten Mineralöl-Kohlenwasserstoffen (MOSH) und aromatischen Mineralöl-Kohlenwasserstoffen (MOAH) mit on-line HPLC-GC-FID

This European Standard was approved by CEN on 10 March 2017.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

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European foreword

This document (EN 16995:2017) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by December 2017, and conflicting national standards shall be withdrawn at the latest by December 2017.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

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1 Scope

This European Standard specifies a highly efficient method for the determination of saturated and aromatic hydrocarbons (from C10 to C50) in vegetable fats and oils and foodstuff on basis of vegetable oils for which it has been interlaboratory validated, with online-HPLC-GC-FID [1], [2] and [3]. This standard is not intended to be applied to other matrices.

The method can be used for the analysis of mineral oil saturated hydrocarbons (MOSH) and/or mineral oil aromatic hydrocarbons (MOAH).

The method has been tested in an interlaboratory study via the analysis of both naturally contaminated and spiked vegetable oil samples and mayonnaise and margarine samples, ranging from 4 mg/kg to 197 mg/kg for MOSH, and from 2 mg/kg to 51 mg/kg for MOAH.

According to the results of the interlaboratory studies, the method has been proven suitable for MOSH and MOAH mass concentrations each above 10 mg/kg.

In case of suspected interferences from natural sources, the fossil origin of the MOSH and MOAH fraction can be verified by examination of the pattern by GC-MS.

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.

EN ISO 661, *Animal and vegetable fats and oils - Preparation of test sample (ISO 661)*

3 Terms and definitions

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

3.1
mineral oil saturated hydrocarbons
MOSH
paraffinic (open-chain, usually branched) and naphthenic (cyclic, alkylated) hydrocarbons

3.2
mineral oil aromatic hydrocarbons
MOAH
aromatic mainly alkylated hydrocarbons

3.3
unresolved complex mixture
UCM
complex mixture of saturated or aromatic hydrocarbons not resolved by gas chromatography such as branched paraffins, alkylated naphthenes and alkylated aromatics

4 Principle

The fractions of MOSH and MOAH are isolated and separated by an HPLC-GC-FID system. MOSH and MOAH fractions are separated on a silica gel column using a *n*-hexane/dichloromethane gradient and each transferred as 450 µl fractions to GC using the Y-interface [4], while triglycerides are kept on the HPLC column. Solvent vapours are discharged via a solvent vapour exit located between the uncoated pre-column and the GC separation column. Volatile components are retained by solvent trapping

applying partially concurrent eluent evaporation. High boiling components spread over the entire length of the flooded zone and are refocused by the retention gap technique [2].

The area attributed to mineral oil is calculated by subtraction of sharp peaks due to n-alkanes (naturally occurring hydrocarbons), terpenes, squalene and its isomerization products, sterenes and olefins with the structure of carotenoids. MOSH and MOAH are quantitated by internal standard added before analysis. Verification standards are added for monitoring proper HPLC fractionation and GC transfer conditions.

Some vegetable oils contain odd-numbered n-alkanes in the range of C21-C33 in such quantities that the chromatograms of the MOSH fraction are severely overloaded and that they might overlap with the mineral oil hump. In this case, it is recommended to use an additional clean-up technique. Activated aluminium oxide strongly retains long chain n-alkanes. Mineral oil which contaminates edible oil almost exclusively consists of branched and cyclic components which are not retained by activated aluminium oxide. Therefore, the use of activated aluminium oxide enables the removal of plant paraffins.

Epoxidation is a purification step that is necessary for the quantification of MOAH. This purification step allows the elimination of olefins like squalene, which elute within the MOAH fraction and interfere with quantification (e.g. olive oil, palm oil). Epoxidation also removes certain olefins co-eluting with the MOSH fraction, therefore epoxidation also may be used as a purification step for the MOSH fraction. Since now, the epoxidation step is the best compromise to remove olefins even though it is not fully quantitative and the efficiency may be sample dependent. Depending on the sample, this reaction may induce the epoxidation of a part of the MOAH or incomplete removal of the interfering olefins.

5 Reagents

Unless otherwise specified, use only reagents of recognized analytical grade.

5.1 Silica Gel 60[1)]**,** extra pure for column chromatography with particle size from 60 µm to 200 µm (70 mesh to 230 mesh) in glass bottle to prevent contamination.

5.2 Silica Gel 60, activated.

Condition silica gel (5.1) in an oven for at least 16 h at 400 °C.

5.3 Demineralized water.

5.4 Anhydrous sodium sulfate, analytical grade, purity ≥ 99 %.

5.5 *n*-Hexane, trace organic analysis grade, for pesticide residue analysis.

n-Hexane purity can be checked by concentrating 30 ml of *n*-hexane mixed with 25 µl of internal standard solution (5.21) and 2 drops of bis(2-ethylhexyl) maleate (5.29) using a rotary evaporator, dissolving the residue in 0,2 ml of *n*-hexane and the analysis of 50 µl by online-HPLC-GC-FID (6.8). Take care that in the evaporation step the residue is not evaporated to dryness to avoid loss of volatile hydrocarbons. The signal abundance of the residue after evaporation should not exceed a tenth of the signal abundance obtained at the quantification limit.

1) Silica gel is available from Merck, reference 7754 or 7734 (www.merck-chemicals.com). It is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

5.6 Dichloromethane (DCM), trace organic analysis grade, purity ≥ 99 %.

DCM purity can be checked by concentrating 50 ml of DCM mixed with 25 µl of internal standard solution (5.21) and 2 drops of bis(2-ethylhexyl) maleate (5.29) using a rotary evaporator, dissolving the residue in 0,2 ml of *n*-hexane and the analysis of 50 µl by online-HPLC-GC-FID (6.8). Take care that in the evaporation step the residue is not evaporated to dryness to avoid loss of volatile hydrocarbons. The signal abundance of the residue after evaporation should not exceed a fifth of the signal abundance obtained at the quantification limit.

5.7 Dichloromethane solution.

Mix 30 ml DCM (5.6) with *n*-hexane (5.5) up to a volume of 100 ml. The solution should be freshly prepared daily.

5.8 Toluene.

5.9 1,1,2-Trichloroethane.

5.10 Perylene (Per), purity ≥ 99 %.

5.11 5-*a*-Cholestane (Cho), purity ≥ 97 %.

5.12 *n*-Undecane (*n*-C11), purity ≥ 98 %.

5.13 *n*-Tridecane (*n*-C13), purity ≥ 97 %.

5.14 Tri-tert-butylbenzene (TBB).

5.15 Bicyclohexyl (CyCy), purity ≥ 99 %.

5.16 1-Methylnaphthalene (1-MN), purity ≥ 95 %.

5.17 2-Methylnaphthalene (2-MN), purity ≥ 97 %.

5.18 Pentylbenzene (PB), purity ≥ 96 %.

5.19 Stock solutions, mass concentration ρ = 10 mg/ml.

Prepare individual stock solutions by weighing, to the nearest 1 mg, 100 mg of *n*-C11 (5.12), *n*-C13 (5.13), TBB (5.14), CyCy (5.15), 1-MN (5.16), 2-MN (5.17) and PB (5.18) into a 10 ml volumetric flask and dilute to the mark with 1,1,2-trichloroethane (5.9) or toluene (5.8). Store the solutions at room temperature. If crystals precipitate during storage, warm the solution until everything has dissolved.

5.20 Internal standard solution 1 (ISTD1)[2)]**.**

Weigh, to the nearest 0,5 mg, 12 mg of Per (5.10) and Cho (5.11) in a volumetric flask of 20 ml (6.22), to which 600 µl of each stock solution (5.19) is added with the exception of *n*-C13, of which 300 µl is added. Fill the volumetric flask up to 20 ml with 1,1,2-trichloroethane (5.9) or toluene (5.8). Resulting mass concentrations are for *n*-C13: ρ = 150 µg/ml, for *n*-C11, TBB, CyCy, 1-MN, 2-MN and PB: ρ = 300 µg/ml and for Per, Cho: ρ = 600 µg/ml.

2) This standard mixture is available by e.g. Restek Corp., Cat.# 31070. It is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

5.21 Internal standard solution 2 (ISTD2).

Dilute the ISTD1 solutions by a factor of 30, e.g. 333 µl filled up to 10 ml with *n*-hexane (5.5). Resulting mass concentrations are for *n*-C13: ρ = 5 µg/ml, for *n*-C11, TBB, CyCy, 1-MN, 2-MN and PB: ρ = 10 µg/ml and for Per, Cho: ρ = 20 µg/ml.

5.22 Aluminium oxide 90, alkaline, for column chromatography 0,063 mm to 0,2 mm.

5.23 Aluminium oxide, activated (ALOX).

Condition aluminium oxide 90 (5.22) for at least 16 h at 500 °C in an oven before using.

5.24 Chloroperbenzoic acid (CPBA), purity 70 % to 75 %.

5.25 CPBA solution, ρ = 0,1 g/ml in dichloromethane.

For example 1 g of CPBA (5.24) in 10 ml of DCM (5.6). Clouding of solution does not disturb the reaction. The solution can be used for up to one week.

5.26 Ascorbic acid.

5.27 Silica-ALOX column.

Insert a filter (6.3) in each glass column (6.2). Then, fill in 10 g of ALOX (5.23) and 3 g of silica gel (5.2) and compress.

5.28 Cleanup column.

Insert a filter (6.3) in a glass SPE tube (6.20). Then, fill in 3 g of silica gel (5.2), compress and overlay with 0,5 g of sodium sulfate (5.4).

5.29 Keeper solvent.

The keeper is a solvent that will not evaporate or evaporate to a lesser degree during the evaporation step, e.g. bis(2-ethylhexyl) maleate. A keeper is used to enhance the recovery of volatile compounds.

5.30 Carrier gas for gas chromatography, preferably hydrogen, purity ≥ 99,995 %.

5.31 Auxiliary gases for flame ionization detector, hydrogen, air, and nitrogen suitable for gas chromatography.

5.32 Alkane standard mixture C10 to C40, solution of equal concentration in an apolar solvent, ρ = 1 µg/ml.

5.33 Ethanol, absolute.

NOTE The ethanol purity can be checked by concentrating 50 ml of ethanol mixed with 25 µl of internal standard solution (5.21) using a rotary evaporator, dissolving the residue in 0,2 ml of *n*-hexane and the analysis of 50 µl by online-HPLC-GC-FID (6.8).

5.34 Mixture of ethanol and *n*-hexane, the volume fraction φ is 50 %.

Mix 50 ml of ethanol (5.33) with 50 ml of *n*-hexane (5.5).

5.35 *n*-Pentacontane (*n*-C50), purity ≥ 98 %.