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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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National foreword

This British Standard is the UK implementation of EN 16995:2017.

The UK participation in its preparation was entrusted to Technical Committee AW/275, Food analysis - Horizontal methods.

A list of organizations represented on this committee can be obtained on request to its secretary.

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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

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of an identical text or by endorsement, at the latest by December 2017, and conflicting national standards shall be withdrawn at the latest by December 2017.

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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

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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.

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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.

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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 %.

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5.36 *n*-Pentacontane (*n*-C50) solution in toluene, $\rho \approx 10$ µg/ml.

Weigh 2 mg of C50 (5.35) in a volumetric flask of 20 ml (6.22) and dilute to the mark with toluene (5.8). Proceed to a second dilution of 1 ml in a 10 ml volumetric flask (6.22). Store the solutions at room temperature.

NOTE 1 Solubility of pentacontane in toluene is limited at room temperature. However, the concentration of the solution of pentacontane does not need to be accurate as it is used only to determine the limit of integration for mineral oil peak.

NOTE 2 It is also possible to use a commercial mixture of n-alkanes from C12 to C60 that contains n-pentacontane[3)].

5.37 Sodium carbonate solution, ρ = 0,1 g/ml in water (5.3).

5.38 Mixture of DCM and *n*-hexane.

Mix 20 ml DCM (5.6) with *n*-hexane (5.5) up to a volume of 100 ml. The solution should be freshly prepared daily.

5.39 Blank refined sunflower oil.

6 Apparatus

Usual laboratory apparatus and, in particular, the following. The glassware shall be thoroughly cleaned and rinsed with *n*-hexane (5.5) or baked in an oven before use so that it is free from impurities.

6.1 Centrifuge and centrifuge tubes.

6.2 Glass column for cleanup, 15 cm to 20 cm length and 15 mm to 20 mm internal diameter.

6.3 Filter for glass column.

6.4 Glass vials with screw caps, volume of 40 ml.

6.5 Rotary evaporator, with vacuum and a water bath at 35 °C (recommended).

Care should be taken to prevent cross contamination. Clean the system thoroughly between determinations.

6.6 Automatic evaporator.[4)]

6.7 Glass sample vials, volume of 2 ml.

6.8 High performance liquid chromatograph, coupled with gas chromatograph and flame ionization detector (HPLC-GC-FID).

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3) ASTM D5442 C12-C60 Qualitative Retention Time Mix is available by e.g. Supelco Cat.# 500623. 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.

4) For example, MicroDancer, IR-Dancer (e.g. Zinser) or Syncore Analyst (Büchi). These are examples 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.

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6.9 Data acquisition system, with the possibility of manual integration.

6.10 LC column, e.g. LiChrospher Si 60[5)], 5 µm (250 mm x 2 mm inner diameter (i.d.)) or equivalent.

The silica gel column shall have a capacity to retain 20 mg fat.

6.11 Uncoated precolumn, e.g. Hydroguard® MXT®[5)], 10 m x 0,53 mm or equivalent.

6.12 Capillary column 1, capable for temperatures up to 350 °C.

The column should have the following characteristics: 100 % dimethylpolysiloxane or 95 % dimethyl / 5 % phenyl methylpolysiloxane stationary phase, a length of 15 m, an internal diameter of 0,32 mm or 0,25 mm and a film thickness 0,10 µm to 0,15 µm or equivalent.

6.13 Capillary column 2, from transfer valve to first Y piece, fused silica (FS) methyl silicone deactivated (length 1 m, outside diameter (o.d.) 0,27 mm, inner diameter (i.d.) 0,1 mm).

6.14 Capillary column 3, for hydrogen carrier gas, FS methyl silicone (length 1 m, o.d. 360 µm, i.d. 25 µm).

6.15 Capillary column 4, for solvent vapour exit, FS methyl silicone (length 1 m, o.d. 0,68 mm, i.d. 0,53 mm).

The columns given in 6.13, 6.14 and 6.15 have proven to be suitable for the analysis, however they can be adjusted in accordance with the characteristics of the HPLC-GC apparatus and the analytical conditions.

6.16 Restriction capillary column, transfer valve and solvent vapor exit, FS uncoated (length 1 m, o.d. 360 µm, i.d. 50 µm).

6.17 Microsyringe, 5 µl to 100 µl capacity, suitable for injection in liquid chromatography.

6.18 Analytical balance, reading accuracy 0,000 1 g.

6.19 Pasteur pipette, glass.

The use of plastic Pasteur pipettes and polyethylene film shall be avoided.

6.20 Empty glass column for solid phase extraction (SPE), with glass fibre frits, 6 ml volume.

6.21 Conical flask, volume of 100 ml.

6.22 Volumetric flasks, various sizes.

7 Sample storage

Analyse only samples packed in glass bottles or aluminium foil in order to prevent additional contamination. Plastic and paper packaging are unsuitable.

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5) These are examples 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.

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8 Preparation of the test sample

Prepare the test sample in accordance with EN ISO 661.

9 Preparation of the analytical sample

9.1 Procedure for liquid and solid fats (e.g. sunflower oil, olive oil)

9.1.1 Procedure for liquid and solid fats soluble in *n*-hexane

Weigh, to the nearest 1 mg, 300 mg sample into a 1,5 ml autosampler vial, fill up with *n*-hexane (5.5) and add 30 µl ISTD2 (5.21). Shake the vial well and place it onto the autosampler. Afterwards, measure the solution by online HPLC-GC-FID. The injection volume is 100 µl.

Another possibility is to weigh, to the nearest 1 mg, 300 mg sample into an autosampler vial, to fill it up with 600 µl *n*-hexane and to add 30 µl ISTD2. Shake the vial well and place it onto the autosampler. Afterwards measure the solution by online HPLC-GC-FID. The injection volume is 50 µl.

Depending on the level of contamination, the injected volume may be adapted in order to avoid the overloading of the chromatograms.

The amount of the added internal standards may be increased (e.g. by using 3 µl ISTD1 (5.20)) instead of 30 µl ISTD2, in order to lower the impact of the matrix interferences, if necessary.

If there are strong interferences in the MOSH chromatogram observed by natural, primarily odd-numbered *n*-alkanes in the range of C23 to C33 (see example in Figure A.4 in Annex A), an additional purification of the sample with aluminium oxide is necessary (9.3) before HPLC-GC analysis.

Vegetable oils often contain biogenic olefinic substances (e.g. squalene, terpenes, phytosterenes), which interfere with the chromatography of the MOAH. Remove these substances by epoxidation (9.4) before HPLC-GC analysis.

9.1.2 Procedure for solid fats not soluble in *n*-hexane

If solid fats are not soluble in *n*-hexane, weigh, to the nearest 1 mg, 300 mg sample in a 40 ml glass vial (6.4). Add 30 µl ISTD2 (5.21) and dissolve in 2 ml DCM solution (5.7). Transfer the solution to the cleanup column (5.28). Wash the vial (6.4) first with 1 ml, then again with 1 ml, 2 ml and 10 ml DCM solution (5.7) and apply the solutions to the cleanup column. Elute the hydrocarbon fraction in another 40 ml glass vial. Evaporate the solvent under reduced pressure after addition of 2 drops of bis(2-ethylhexyl) maleate (5.29) at 40 °C. Take care that in the evaporation step the residue is not evaporated to dryness to avoid loss of volatile hydrocarbons. Dissolve the residue of the extract in *n*-hexane (5.5) and transfer it to a vial to a final volume of 1 ml. Centrifugation may be necessary if the solution is cloudy. Analyse the sample solution by online-HPLC-GC-FID. The injection volume is 50 µl.

The amount of the added internal standards may be increased (e.g. by using 3 µl ISTD1 (5.20)) instead of 30 µl ISTD2 (5.21), in order to lower the impact of the matrix interferences, if necessary.

If there are strong interferences in the MOSH chromatogram observed by natural, primarily odd-numbered *n*-alkanes in the range of C23 to C33 (see example in Figure A.4 in Annex A), an additional purification of the sample with aluminium oxide is necessary (9.3) before HPLC-GC analysis.

Vegetable fats often contain biogenic olefinic substances (e.g. squalene, terpenes, phytosterenes), which interfere with the chromatography of the MOAH. Remove these substances by epoxidation (9.4) before HPLC-GC analysis.

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