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Animal and vegetable fats and oils — Determination of aliphatic hydrocarbons in vegetable oils

Corp gras d'origines animale et végétale — Détermination des hydrocarbures aliphatiques en corps gras d'origines végétale

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Foreword

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The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

Introduction

The major saturated hydrocarbons present in vegetable oils are long chain *n*-alkanes, containing more than 21 carbon atoms, and having an odd carbon number preference.[1]

Mineral oils can contain *n*-alkanes with up to 60 carbon atoms with no odd carbon predominance. Chromatograms of mineral oils obtained by this method are characterized by a wide peak due to the presence of a complex mixture of saturated branched and cyclic hydrocarbons. Medium and low viscosity mineral oils are typically characterized by a complex mixture with between C10 and C25 chain length; while high viscosity mineral oils are indicated by a complex mixture with the midpoint around C30 chain length.[2] The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has set several ADIs for mineral oil (2002) dividing low-medium viscosity mineral oils into three different subclasses depending on the point of toxicity. This method does not help to distinguish between different classes.

Chromatograms of diesel oil are characterized by the presence of *n*-alkanes between C10 and C25 chain length with no odd carbon predominance, i.e. both even and odd numbered hydrocarbons are present in relatively equal proportions.

Animal and vegetable fats and oils — Determination of aliphatic hydrocarbons in vegetable oils

1 Scope

This International Standard specifies a method for the determination of saturated aliphatic hydrocarbons from C10 to C56 of natural origin present in vegetable oils, and for detecting the presence of mineral oil and diesel oil.

The method is applicable to all types of crude and refined edible oils and fats, for concentrations of mineral oils from 50 mg/kg to 1 000 mg/kg.

A rapid method for refined and virgin (or cold-pressed) oils is proposed in Annex C. This rapid method is not adapted for crude oils due to a lack of retention of triglycerides observed for some samples.

A method for fat recovery from food samples by soxhlet extraction with a blend of solvents is proposed in Annex D.

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
hydrocarbon contents
sum of saturated aliphatic hydrocarbons, expressed as a mass fraction, determined according to the method specified

3.2
unresolved complex mixture
UCM
complex mixture of saturated hydrocarbons not resolved by gas chromatography, represented by a wide peak, which can be due to a contamination with mineral oil

Note 1 to entry: The width of the peak is approximately 5 min to 15 min depending on gas chromatography conditions,

Note 2 to entry: See relevant chromatograms in Annex A.

3.3
diesel
sum of saturated *n*-alkanes between C10 and C25 chain length, expressed as a mass fraction, determined according to the method

Note 1 to entry: See relevant chromatograms in Annex A.

4 Principle

The saturated aliphatic hydrocarbons of the sample are isolated by liquid chromatography on silica gel impregnated with silver nitrate and determined by capillary gas chromatography with flame ionization detection using an internal standard. From the chromatogram, the area attributed to mineral oil is calculated by the subtraction of sharp peaks due to *n*-alkanes (naturally occurred hydrocarbons) from the total area including the UCM. To indicate diesel contamination, the peak areas of individual hydrocarbons between C10 and C25 chain length are summed and quantified together.

5 Reagents

WARNING — Attention is drawn to national regulations that specify the handling of hazardous substances, and users' obligations thereunder. Technical, organizational and personal safety measures shall be followed.

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

5.1 Silica gel 60[1], extra pure for column chromatography with particle size between 60 µm and 200 µm (70-230 mesh).

5.2 Water, distilled and cooled down to room temperature.

5.3 Anhydrous sodium sulfate, analytical grade, purity 99 % minimum.

NOTE Sodium sulfate may be replaced by sea sand, washed with *n*-hexane.

5.4 *n*-Hexane, trace organic analysis grade, purity 99 % minimum, residue after evaporation maximum 2 mg/kg.

NOTE 1 Hexane purity may be checked by concentrating 200 ml of *n*-hexane mixed with 2 ml of internal standard solution (5.6) using a rotary evaporator, dissolving the residue in 0,2 ml of *n*-hexane and the analysis of 5 µl by gas chromatography (9.3).

NOTE 2 Hexane may be replaced by isooctane, *n*-heptane or a mixture of alkanes of boiling point 65 °C to 70 °C, as long as the residue after evaporation is maximum 2 mg/kg. Solvents with higher boiling point than *n*-hexane take longer to evaporate. However, they are preferred due to the toxicity of hexane.

5.5 Internal standard: *n*-octadecane (C18), purity 99 % minimum.

n-Octadecane may be replaced by *n*-eicosane (C20). Before choosing one of these two compounds as the internal standard, it should be verified that there is no co-elution with other peaks from the sample to be analysed.

n-Octadecane shall be replaced by naphthalene if the sample is contaminated with a diesel oil, in order to avoid the overlapping of the internal standard peak with the alkane peaks to be quantified.

5.6 Solution of internal standard, mass concentration ρ = 0,04 mg/ml.

As an example, weigh to the nearest mg, approximately 50 mg of *n*-octadecane (5.5) and dilute to 25 ml with *n*-hexane (5.4), and then proceed with a second dilution of this mixture of 1 ml → 50 ml with *n*-hexane. Store this solution at room temperature in order to maintain its stability.

5.7 *n*-Decane (C10), purity 99 % minimum.

1) Silica gel is available from Merck, reference 7754 or 7734. This reference is an example of a suitable product which is available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

5.8 *n*-Decane solution, mass concentration ρ = 0,04 mg/ml.

As an example, weigh to the nearest mg, approximately 50 mg of *n*-decane and dilute to 25 ml with *n*-hexane (5.4), and then proceed with a second dilution of this mixture of 1 ml → 50 ml with *n*-hexane. Store this solution at room temperature in order to maintain its stability.

5.9 Octatetracontane (C48), purity 99 % minimum. This standard is used to limit the integration of the hump to a certain retention time that will correspond to the retention time of this hydrocarbon.

5.10 Octatetracontane solution, mass concentration approximately ρ = 0,08 mg/ml.

As an example, weigh to the nearest mg approximately 2 mg of octatetracontane (5.9) and dilute to 25 ml of *n*-hexane (5.4). Store this solution at room temperature in order to maintain its stability.

NOTE Solubility of octatetracontane in hexane is limited at room temperature, due to its high melting point. However, the concentration of the solution of octatetracontane does not need to be accurate as it is used only to determine the limit of integration for the mineral oil peak.

5.11 Silver nitrate ($AgNO_3$), analytical grade.

5.12 Silver nitrate aqueous solution, mass concentration ρ = 0,75 g/ml.

As an example, to prepare silver nitrate silica gel for 3 columns, weigh approximately 4,5 g of silver nitrate in 6 ml of distilled water (5.2).

5.13 Carrier gas for gas chromatography, helium or hydrogen.

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

5.15 Alkane standard mixture C10 to C40[2], solution in non-polar solvent.

5.16 Viscous paraffin and highly liquid paraffin[3], solution in non-polar solvent.

5.17 Solution of paraffin and *n*-octadecane, mass concentration of paraffin ρ = 0,5 mg/ml, mass concentration of *n*-octadecane ρ = 0,08 mg/ml.

As an example, weigh to the nearest mg, approximately 500 mg of viscous paraffin (5.16) and 80 mg of *n*-octadecane (5.5) and dilute to 10 ml with *n*-hexane (5.4), and then proceed with a second dilution of this mixture of 1 ml → 100 ml with *n*-hexane. Store this solution at room temperature in order to maintain its stability.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

IMPORTANT — The glassware used for the determination shall be thoroughly cleaned and rinsed with n-hexane (5.4) before use so that it is free from impurities.

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2) Alkane standard mixture at 50 mg/l is available from Sigma-Aldrich, reference 68281 (www.sigmaaldrich.com). This reference is an example of suitable products which are available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

3) A viscous paraffin is available from Merck, reference 107160. Highly liquid paraffin is available from Merck, reference 107174. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

6.1 Glass column for chromatography (30 cm to 40 cm length and 15 mm to 20 mm internal diameter), fitted with sintered glass discs and polytetrafluoroethylene (PTFE) stop cock.

NOTE A pad of cotton wool exhaustively extracted with *n*-hexane may be used to replace the sintered glass discs in the glass column.

6.2 Glass rods.

6.3 Round-bottomed flasks, 250 ml and 500 ml capacity.

6.4 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.

6.5 Automatic evaporator[4], for 10 ml tube (optional), recommended operating conditions: temperature of water bath = 35 °C, nitrogen pressure = 5 psi.

6.6 Conical glass sample vials, 10 ml capacity.

6.7 Gas chromatograph, suitable for use with capillary column, equipped with an on-column injector or equivalent device, a temperature-programmable oven and a flame ionization detector (FID).

NOTE A programmed temperature vaporization injector (PTV) may also be used.

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

6.9 Capillary column, capable of being programmed up to 400 °C ("high temperature" type) for which the following characteristics are recommended: 100 % dimethylpolysiloxane or 95 % dimethyl/5 % diphenyl polysiloxane stationary phase, length 15 m, internal diameter 0,32 mm or 0,25 mm, film thickness 0,1 µm.

NOTE In order to get a separation between the solvent peak and mineral oil containing short chain hydrocarbons (C10 to C14), a 30 m long capillary column can be used.

6.10 Microsyringe, 5 µl to 10 µl capacity, suitable for on-column injection in gas chromatography.

6.11 Analytical balance, reading accuracy 0,001 g, weighing precision 0,001 g.

6.12 Pasteur pipette, in glass.

Plastic Pasteur pipettes shall be avoided. Polyethylene film shall also be avoided.

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.[3]

4) Zymark TurboVap LV evaporator is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

8 Preparation of the test sample

Prepare the test sample in accordance with ISO 661.

9 Procedure

9.1 Chromatography column preparation

9.1.1 Preparation of $AgNO_3$ impregnated silica gel

Preparation of the silver nitrate silica gel column (for 3 columns): weigh 45 g of silica gel (5.1) in a 500 ml round-bottomed flask (6.3) protected by aluminium foil. With a Pasteur pipette (6.12), add drop by drop the silver nitrate solution (5.12) shaking continuously. Shake well for 30 min to homogenize. After completing, cover the flask with aluminium foil and allow to stand at room temperature for 12 h before use.

In order to improve homogenization, it is recommended to use an automatic shaker. If no automatic shaker is available, it is possible to put the flask in a rotatory evaporator equipment and rotate for 30 min without vacuum.

NOTE 1 The impregnated silica gel can be stored one week at room temperature in a desiccator, provided the flask is protected with aluminium foil.

NOTE 2 For screening purposes, $AgNO_3$ impregnated silica gel can be replaced by non-impregnated silica gel. The results will be similar or higher than those obtained using silvered silica gel.

NOTE 3 For refined vegetable oils other than refined olive pomace oil, $AgNO_3$ impregnated silica gel can be replaced by non-impregnated silica gel.

9.1.2 Column packing

In a beaker, suspend 18,5 g of silver nitrate impregnated silica gel (9.1.1) in *n*-hexane (5.4). The slurry is introduced onto the column (6.1) containing 40 ml of *n*-hexane and pack the column by tapping it gently using a glass rod (6.2). Add at least 0,5 cm to 1 cm of sodium sulfate (5.3) on top of the $AgNO_3$ - silica gel, and compress the $AgNO_3$ - silica gel bed with a stream of nitrogen. Rinse the $AgNO_3$ - silica gel with another 60 ml of *n*-hexane (5.4) to eliminate impurities in the $AgNO_3$ - silica gel.

The column should be covered with a black paper cylinder or with aluminium foil to avoid oxidation of the silver nitrate.

Elute the solvent until the level of the solvent in the column is about 0,5 cm higher than the $AgNO_3$ - silica gel bed. Put a 250 ml round-bottomed flask (6.3) under the chromatography column.

9.2 Elution of the hydrocarbon fraction

Weigh to the nearest 1 mg, 1 g of the sample in a beaker and add 1 ml of the solution of the internal standard (5.6), transfer the solution to the chromatographic column (9.1.2) with the aid of a Pasteur pipette (6.12) and let the sample penetrate into the stationary phase. Wash the beaker with two portions of 1 ml of *n*-hexane (5.4) and introduce the solution into the column. Elute the hydrocarbon fraction with 55 ml of *n*-hexane (5.4) with a cadence of approximately 15 drops every 10 s, collecting the fraction in a 250 ml flask (6.3). Evaporate most of the solvent up to 1 ml or 2 ml with a rotary evaporator equipped with a water bath set to 35 °C (6.4). Transfer the concentrated solution to a 10 ml conical tube. Concentrate the solvent up to 0,5 ml from the conical tube under a stream of nitrogen, using either a water bath at 35 °C or an automatic evaporator (6.5). Take care that, in both evaporation steps, the residue is not evaporated to dryness to avoid loss of the volatile alkanes.

Adjust the elution volume of *n*-hexane by the analysis of 1 ml solution of paraffin (5.17) and collecting consecutive fractions of 50 ml, 10 ml, and 10 ml, and analysing each one by gas chromatography (GC).

9.3 Gas chromatography

9.3.1 Gas chromatography setup

Install the column (6.9) in the gas chromatograph (6.7) and check the working conditions by injecting the solvent, *n*-hexane (5.4). The baseline should be straight with a small positive drift. If the drift is high, proceed to condition the column, for a negative drift check the connections of the column.

If the column is used for the first time, it is necessary to condition the column by heating it in the column oven using a temperature gradient up to 370 °C (depending on the oven temperature chosen for the analysis) in 4 h. Maintain the temperature for 2 h.

9.3.2 Working conditions for gas chromatography analysis

The following working conditions have proved satisfactory for the analysis:

Column	DB5 HT (15 m long - 0,25 mm internal diameter - 0,10 µm film thickness)
Oven temperature	Initial temperature 60 °C for 3 min, programmed at 12 °C/min to 350 °C, hold for 10 min
Carrier gas	Hydrogen head pressure of 100 kPa
Detector temperature	370 °C
Injection volume	2 µl

NOTE When a programmed temperature vaporization injector is used, the following working conditions have proved to be satisfactory for the analysis: initial temperature 50 °C for 0,5 min; programmed at 300 °C/min to 300 °C, hold for 10 min.

These conditions may be adjusted in accordance with the characteristics of the gas chromatograph apparatus and the column. **However, the oven temperature shall be brought up to 350 °C in order to elute the high molecular weight hydrocarbons.** A temperature ramp of 12 °C/min is a good compromise between a good sensitivity due to a "thinner hump" and a limited baseline drift.

Typical chromatograms are presented in Annex A.

9.3.3 Peak identification

Identify the internal standard *n*-octadecane by injecting 2 µl of the standard solution (5.6). Check the resolution of the *n*-decane (C10) separated from the solvent peak, by injecting 2 µl of the standard solution (5.8). The resolution of the octatetracontane (C48) can be checked by injecting 2 µl of the standard solution (5.10). See the chromatograms in Figures A.1 and A.2.

Inject 2 µl of the alkane standard mixture C10-C40 (5.15) in order to identify the areas to take into consideration for the calculation of C10-C25 alkane concentration (Figure A.1).

In sunflower oils, the major peaks correspond to saturated aliphatic hydrocarbons C27, C29 and C31 (Figure A3).

The resolution of highly liquid paraffin and viscous paraffin (5.16) can be checked by injecting 2 µl of the 0,5 mg/ml standard solution (Figures A.4 and A.5).

A broad peak of about 5 min to 15 min width, depending on the GC conditions, represents a complex mixture of hydrocarbons (UCM) that the chromatography cannot resolve and is attributed to mineral oils (Figure A.6 to Figure A.10).

In case of contamination of vegetable oil with diesel oil, the chromatogram is characterized by the presence of *n*-alkanes between C10 and C25 chain length with no odd carbon predominance (Figure A.11). Naphthalene shall then be used as internal standard.