Preprint. Food Chemistry 480 (2025) 143880. DOI: 10.1016/j.foodchem.2025.143880

STUDY ON THE CONTENT AND PROFILE OF MOSH AND MOAH IN UNPROCESSED MEAT BY LC/GC×GC-FID/MS

Paula Albendea[1], Giorgia Purcaro*,[1]

[1] Analytical Chemistry Lab, Gembloux Agro-Bio Tech, University of Liège, Gembloux, 5030, Belgium

* Corresponding author. Contact information:

Giorgia Purcaro, gpurcaro@uliege.be

Gembloux Agro-Bio Tech, University of Liège

Bât. G1 Chimie des agro-biosystèmes, Passage des Déportés 2,

5030 Gembloux, Belgium

Office phone: +32 (0)81 62 22 20

Abbreviations: Cho, Cholestane; Cycy, cyclohexyl-cyclohexane; EtOH, ethanol; IS, Internal standard; LC-GC×GC-FID/MS, liquid chromatography-gas chromatography-flame ionization detector and liquid chromatography-comprehensive multidimensional gas chromatography-flame ionization detector/mass spectrometry; LC-GC-FID, liquid-gas chromatography-flame ionization detection; mCPBA, m-Chloroperbenzoic acid; MeOH, methanol; MN, methyl naphthalene; MASE, microwave-assisted saponification and extraction; MOH, Mineral oil hydrocarbons; MOAH, mineral oil aromatic hydrocarbons; MOSH, Mineral oil saturated hydrocarbons; 5B, pentylbenzene; Per, perylene; TBB, tri-tert butyl benzene.

Abstract

Mineral oil hydrocarbons (MOH) contamination was evaluated in 30 samples of various unprocessed meats. Preliminary tests showed that a saponification method with a 2 M KOH solution was the most adequate for sample treatment for meat. Most of the meat samples showed saturated MOH (MOSH) in a carbon chain length range that could have accumulated by the animals. However, the highest contamination found in two beef rib samples occurred probably during slaughter or handling prior to commercialization. This was suggested by the MOSH profile (i.e. high molecular weight), the presence of aromatic MOH (MOAH), hopanes and alkylbenzenes. The primary type of MOSH found in the meat was generally paraffins, and the MOAH in beef ribs were exclusively composed of 1-2 ring compounds. These findings contribute to a comprehensive understanding of the types of MOH that can be found in animal food products, addressing a gap in the existing literature.

Keywords: Mineral oil saturated hydrocarbons; Mineral oil aromatic hydrocarbons; LC-GC-FID; LC-GC×GC-FID/MS; meat; saponification

1. Introduction

Mineral oil hydrocarbons (MOH) are environmental, and processing contaminants composed of a complex mixture of liposoluble compounds of petrogenic origin, containing between 10 and 50 carbon atoms (Bratinova et al., 2023). These contaminants can be classified into two main categories based on their chemical structure, named mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH). Each of these categories can be further subdivided into various subclasses. In the MOAH fraction, the distinction is based on the number of aromatic rings. Moreover, carcinogenic, genotoxic, and mutagenic effects have been associated with compounds with three to seven aromatic rings (EFSA, 2012, 2019, 2023). In the case of MOSH, a distinction can be made between paraffins (linear and branched alkanes) and naphthenes (alkyl-substituted cycloalkanes). Current toxicological data suggest that MOSH can accumulate in animal and human organs and tissues, with this accumulation being influenced by the carbon chain length (generally C_{16} - C_{35}) and structural characteristics of the hydrocarbons (EFSA, 2012, 2023). Although MOSH exhibit no concern for human health, the consumption of animal products that have accumulated specific sub-classes of MOSH may increase the selective human exposure to the most prone to accumulate, leading to potential health risks (EFSA, 2023). Specifically, an unresolved cloud of mostly highly branched alkanes and alkylated cycloalkanes was found to accumulate in different rat tissues (liver and spleen) and humans (liver and adipose tissue) (Barp et al., 2017; Biedermann et al., 2015; Cravedi et al., 2017; Isola et al., 2023).

The current data on MOSH in animal-origin food products is mainly limited to animal fats intended for human consumption and eggs (EFSA, 2012). Only two papers reported data on meat. Six samples were evaluated among hundreds of different food products in a Belgian market survey (Van Heyst et al., 2018), covering meat from beef and chicken, whereas MOSH levels of pork loin from animals fed with different diets were studied in Albendea et al. (2024). To address this research gap, this study targeted the analysis of meat samples coming from different animals. Only unprocessed meat samples were considered in order to reduce the possible MOH sources of contamination and facilitate the evaluation of the contribution of MOSH accumulation in animal tissues to the final contamination of the meat.

The reference method for routine analysis of MOH is hyphenated liquid-gas chromatography-flame ionization detection (LC-GC-FID), applying a method developed by Biedermann et al. in 2009 and further optimized (Barp et al., 2013; Biedermann et al., 2009, 2017). In this system, the purpose of the LC is to isolate the MOH mixture from other lipid compounds and to separate it into MOSH and MOAH fractions, which are then independently transferred to the GC for further separation based on their volatility. The use of LC-GC-FID enables the quantification of the total amounts of MOSH and MOAH; however, it provides limited information about their chemical structures, offering only the distribution by carbon chain length. Consequently, the growing interest in studying the different subclasses of MOSH and MOAH has led to the use of even more advanced techniques. Specifically, the integration of a second dimension in GC

(i.e., comprehensive two-dimensional GC, GC×GC), that simplify the interpretation of the results thanks to clearly structured chromatograms (Bauwens et al., 2023a), and the inclusion of a mass spectrometer (MS) for confirmation purposes in the analytical system has allowed for a more detailed characterization of MOH contamination.

A critical step in MOH analysis is the sample preparation required before LC separation, which aims to remove potential interferences and enrich the MOH fraction to enhance the sensitivity. The steps involved in the sample preparation can vary depending on the type of food matrix. However, saponification is commonly performed to remove triglycerides, which would otherwise limit the amount of sample that can be injected into the LC column, reducing the sensitivity (Biedermann & Grob, 2012). Nevertheless, this step can heavily influence the distribution of the internal standards (ISs) used to control the performance of the different steps and for the final quantification of MOSH and MOAH. The reliability of the results is ensured when the specific ratios between the different ISs are maintained. Saponification impacts mainly the distribution of tri-tert butyl benzene (TBB) and methyl naphthalenes (MNs) (which should keep a ratio of 1.0) across different solvent phases (Bratinova et al., 2023; Menegoz Ursol et al., 2022). An alternative saponification procedure was recently proposed to improve this aspect in edible oils and fats (Bauwens & Purcaro, 2024).

The aim of this work is to evaluate the MOH contamination present in different types of commonly consumed unprocessed meat, focusing the attention on the composition in MOSH and MOAH subclasses, as recommended by EFSA in the

latest published scientific opinion (EFSA, 2023). For that purpose, the previously optimized saponification method by Bauwens & Purcaro (2024) was adapted and validated. The final analysis was performed using the validated LC-GC×GC-FID/MS platform (Bauwens, et al., 2023a; Bauwens, et al., 2023b) to obtain detailed information on the sub-classes of MOH present in the samples.

2. Material and methods

2.1. Reagents and standard solutions

m-Chloroperbenzoic acid (mCPBA), methanol (MeOH) HPLC grade, sodium thiosulfate ($Na_2S_2O_3$), sodium sulfate, sodium carbonate ($NaCO_3$), bis(2-ethylhexyl) sebacate and dichloromethane LiChrosolv® were provided by Merck-MilliporeSigma (Darmstadt, Germany). Acetone and *n*-hexane HPLC grades were purchased from Biosolve Chemicals (Dieuze, France) and the last one was distilled before use. Ethanol 99.8% (EtOH) for HPLC was from Thermo Scientific (Waltham, MI, USA) and milli-Q water was obtained with a Millipore system (Bedford, MA, USA). All solvents were tested for purity before use.

A standard mixture of *n*-alkanes in cyclohexane, which contained even-numbered alkanes in the range $C_{10\text{-}50}$ at 100 mg/L each (#31076), was used to verify GC performance. The IS solution used as a quality control of the analytical method and to quantify MOH (#31070) contained *n*-C13 at 0.15 mg/mL, TBB, *n*-C11, CyCy (cyclohexyl-cyclohexane), 5B (pentylbenzene), 1-MN and 2-MN at 0.30 mg/mL, and Cho (cholestane) and Per (perylene) at 0.60 mg/mL in toluene. Both standard solutions were kindly provided by Restek (Neukirchen-Vlun, Germany)

and stored at −18 °C. A motor oil (Sternel) was used for the recovery test and the sensitivity evaluation. This motor oil has a similar level of MOSH and MOAH (44± 1% and 56± 1%, respectively) and a distribution between *n*-C_{20} and *n*-C_{36}, centered on *n*-$C_{26,}$

2.2. Samples and samples homogenization

Five different types of unprocessed meat were targeted in this study: bacon, pig rib, beef rib, turkey breast, and dark chicken meat (entire legs with skin). In this way, a variety of animal species with different digestive systems were covered, considering two monogastric species (pig and poultry) and one ruminant (beef). The pieces of meat studied were chosen considering their lipid content and their availability in the market.

A total of 30 samples of meat (6 samples per type) were purchased from different local supermarkets and butcher shops in Wallonia (Belgium). Each sample was deboned, homogenized with an IKA® M 20 universal mill (IKA-Werke GmbH & Co. KG, Staufen, Germany), wrapped in aluminum foil and conserved inside zip bags at −18 °C until analysis. All the materials used for sample preparation, including the homogenizer, were rinsed with acetone and hexane before their use.

2.3. Sample preparation method

2.3.1. Saponification and extraction

The microwave-assisted saponification and extraction (MASE) methods were performed in an ETHOS X system equipped with an SR-12 eT TFM rotor

(Milestone Srl, Bergamo, Italy) and 5 g of homogenized sample was always used. Two different procedures were compared in 3 different samples (bacon, pig rib, and beef rib):

Saponification method with saturated methanolic KOH solution (named Sap MeOH): This MASE method was applied as described in Albendea et al. (2024) for pig loin. Briefly, after weighing the sample inside a microwave Teflon vessel, 10 mL of n-hexane and 10 µL of IS were added, followed by 10 mL of a saturated methanolic KOH solution. The MASE was performed at 120 °C for 20 min, with 5 min pre-heating. Once the vessels reached the ambient temperature, 40 mL of water and 3 mL of methanol were added along the walls, and the vessels were kept at −18 °C for 30 min. When the vessels were at room temperature, the upper phase was recovered quantitatively and concentrated to 4 mL. Then, the concentrated phase was subjected to a washing step with a solution of MeOH:H_2O (2:1; v/v) and the final organic phase was obtained. The final organic phase collected was concentrated to 500 µL after adding bis(2-ethylhexyl) sebacate as a keeper. Finally, a spatula tip of sodium sulfate was added to remove possible residual water before the LC separation.

Saponification methods with 2M KOH solution in EtOH:H_2O (1:1;v:v) (named Sap EtOH:H_2O): The conditions tested were adapted from the method recently published by Bawens and Purcaro for edible oils (Bauwens & Purcaro, 2024). 10 mL of n-hexane, 10 µL of IS, and 10 mL of a 2M KOH solution in EtOH:H_2O (1:1;v:v) were added to the Teflon vessel with the sample and two different

microwave conditions were tested. As proposed in Bauwens & Purcaro (2024), a MASE for 30 min at 60 °C with 5 min of pre-heating was carried out, whereas the other conditions tested were 120 °C for 20 min also with 5 min of pre-heating. After the MASE, 20 mL of H_2O and 0.5 mL of EtOH were added along the walls of the vessels and were kept in the fridge for 20 min. Then, the hexane phase was recovered, and a second extraction was performed with 5 mL of *n*-hexane in the microwave vessel. After stirring for 5 min, 0.5 mL of EtOH was added to obtain the phase separation. The upper layer was transferred to the tube with the previous extract and concentrated to 4 mL. A washing step with 4 mL of H_2O was performed to obtain the final organic phase. The final organic phase collected was concentrated to 500 µL after adding bis(2-ethylhexyl) sebacate as a keeper. Finally, a spatula tip of sodium sulfate was added to remove possible residual water before the LC separation.

2.3.2. Epoxidation

The epoxidation was performed according to the protocol validated in ISO/DIS 20122 (2024). Briefly, 1 mL of a mCPBA solution (100 g/L in EtOH) was added and, after stirring the mixture for 20 min at 40 °C, 500 µL of ethanol and 2 mL of deactivation solution (50 g/L of $Na_2S_2O_3$ and 50 g/L of $NaCO_3$ in H_2O) was added, and the vial was shaken for about 1 min at about 750 rpm to deactivate any excess mCPBA. The upper hexane phase was transferred to a new sample vial, and a spatula tip of sodium sulfate was added to remove possible residual water before the injection in LC.

2.3.3. Verification of the methods

The meat sample with the lowest MOH contamination (beef rib) was chosen to evaluate the MOSH and MOAH recovery of the MASE methods. Thus, this sample was spiked at two different levels with Sternel motor oil and four replicates per each level were subjected to each MASE. MOH were added in 3.2 mg/kg (corresponding to 1.4 mg/kg of MOSH and 1.8 mg/kg of MOAH) for the lowest spiked level, whereas 7.2 mg/kg (corresponding to 3.2 mg/kg of MOSH and 4 mg/kg of MOAH) were added for the highest one.

After applying the MASE methods previously described, bis(2-ethylhexyl) sebacate was added as a keeper to the final organic phase collected. Then, it was concentrated to 1 mL and subjected to the epoxidation reaction to eliminate MOAH interferences (mostly squalene).

The linearity and the sensitivity of the analytical instrument in 1D and 2D mode (GC×GC) were evaluated. A six-point calibration curve was prepared using Sternel motor oil to cover the range from approximately 10 ng to 2000 ng of MOH injected into the GC system. Each calibration point was prepared in triplicate.

The regression curves were obtained by applying the least squares method, and the linearity within the range considered was assessed using visual residue analysis.

The limit of quantification (LOQ) was estimated using the standard deviation obtained from 10 blanks of the analytical procedure injected into the system in

1D mode and 2D mode (by switching off and on the modulator and adapting the oven program).

2.4. Mineral oil hydrocarbons determination

The determination of MOH was performed in a LC/GC(×GC)-FID/MS system composed with the following modules:

1) A CHRONECT Robotic RTC autosampler (Axel Semrau GmbH, Sprockhövel, Germany) was connected to the LC and GC modules. Thus, it performed the injections in both systems plus the collection of MOSH and MOAH fractions after LC separation. The autosampler was controlled by the software Chronos (Axel Semrau GmbH, Sprockhövel, Germany).

2) An Agilent 1260 Infinity II LC equipped with a binary pump and a Variable Wavelength Detector acquiring at 230 nm (Agilent Technologies, Waldbronn, Germany). The pump was modified by Axel Semrau to ensure the minimization of dead volumes. The LC system was equipped with an Allure silica column of 25 cm × 2.1 mm i.d. packed with Si-60, 5 µm particle size (Restek) and controlled by the software Clarity™ (DataApex, Prague, Czech Republic). The elution of MOH in LC was performed with a flow of 0.3 mL/min and the gradient program was as follows: 0 min 100% *n*-hexane; 1.5-6 min 65% *n*-hexane and 35% of DCM. At 6.10 min, the column was backflushed with 100% DCM for 9 min at 0.5 mL/min and then reconditioned with 100% of *n*-hexane for 10 min at 0.5 mL/min and for 5 min at 0.3 mL/min. The exit of the LC detector was connected through a

transfer valve (VICI AG International, Schenkon, Switzerland) to guide the LC eluent into a collection vial. The MOSH fraction was collected between 2 and 3.5 min and the MOAH between 4.4 and 5.9 min (corresponding to 450 µL each). For each sample analyzed in this study,100 µL of the concentrated hexane phase obtained after MASE were injected into the LC to collect MOSH and MOAH in different vials. This step was performed three times per each analytical replicate. Thus, at the end of the three LC collections, 1,350 µL (450 µL x 3 colllections) were obtained for each fraction and a concentration step under N_2 steam was applied until a volume of ~70 µL. In this way, it was injected into the GC system a comparable amount that a single online transfer.

3) The GC and GC×GC system consisted of a Pegasus BT 4D GC×GC time-of-flight (ToF) MS (LECO, St. Joseph, MI, USA) and controlled by the software ChromaTOF BT Version 5. The system was constituted by an Agilent 7890A gas chromatograph, equipped with a secondary oven and a quad-jet dual-stage thermal modulator, an FID detector and a ToF MS. For the FID line, an on-column injector was used, and an Rxi-retention gap was installed (4 m × 0.53 mm i.d) before the set of GC columns, whereas the MS line was equipped with a multimode injector (MMI), used in spitless mode and at 300 °C. Both lines (to the FID and the MS) were equipped with a Rxi-17Sil MS (15 m × 0.25 mm i.d. × 0.25 mm) column connected to a Rxi-1 MS HT (0.6 m × 0.15 mm i.d. × 0.15 µm) column, provided by Restek. The carrier gas, helium, was supplied in constant flow mode at 1.7

mL/min to the FID and 1.3 mL/min to the MS. For the 1D analysis, the GC oven temperature program was: 60 °C (hold 8 min) to 350 °C (hold 5 min) at 20 °C/min. For the 2D analysis, the GC oven temperature program of the primary oven was 59 °C (hold 5 min) to 350 °C (hold 5 min) at 5 °C/min. Regardless of the analysis mode (1D or 2D), a 15 °C positive offset was applied for the modulator and 5 °C for the secondary oven. The modulator was switched off to operate in 1D mode. Modulation was performed every 6 s, applying hot and cold pulses of 1.80 s and 1.20 s of duration, respectively. The FID was set at 370 °C and operated using 40 mL/min of H_2, 400 mL/min of air flow and 30 mL/min of the make-up gas (N_2). For the MS, the mass range was between 40 m/z and 700 m/z; the temperatures used for the MS interface and ion source were 330 °C and 250 °C, respectively and the electron ionization (EI) was used at 70 eV. Spectra generation frequency in both detectors in 2D was 200 Hz for FID and 100 Hz for MS and in 1D 20 Hz for both detectors.

2.5. Integration and calculations

Data was acquired and elaborated using the software ChromaTOF BT Version 5 for MOSH/MOAH. The integration and all the calculations were performed following the JRC guidance (Bratinova et al., 2023; Bratinova & Hoekstra, 2019). The MOSH and MOAH areas were determined in GC and GC×GC by trimming all the interferences from the whole hump of the largely unresolved peaks with automatic smooths applied with the software. The position of the baseline was corrected for both types of MOH by the subtraction of the signal of blanks

obtained on the same day and by using a constant in case of baseline shift between the blanks and the samples. For the estimation of the different MOSH and MOAH subclasses, classifications were created based on the position of the compounds in the GC×GC chromatogram, based on the 2D structure obtained from the analysis of well-characterized motor oil and technical white oil (**Fig S.2** in supplementary materials), supported by the information obtained by the MS about the molecular structure, and the data available in the literature (Carrillo et al., 2022a; Biedermann et al., 2015).

The ratio between the IS peak area was used for quality control. The quantification of MOH was carried out using the IS method, Cycy for MOSH and TBB for MOAH, and the results were expressed as concentrations in the unprocessed meat (mg/kg).

3. Results and discussion

3.1. Optimization and verification of the saponification method

The only saponification method available in the literature applied to meat was the one recently published by Albendea et al. (2024) for pig loin, where a saturated KOH solution in MeOH was used and the reaction was performed at 120°C for 20 min. Nevertheless, using saturated KOH in MeOH raised problems for the ISs ratio (i.e., TBB/2-MN) in edible oils (Bauwens & Purcaro, 2024; ISO/DIS 20122, 2024; Menegoz Ursol et al., 2022), as thoroughly discussed by Bauwens & Purcaro (2024). To solve this issue, in the latter reference, different concentrations of KOH, solvent mixtures and temperatures for the saponification were tested. Using a 2M KOH solution in EtOH:H_2O (1:1;v:v) for edible oils, a

TBB/2-MN ratio of 1.05 ± 0.01 was obtained, which was significantly closer to the expected ratio of 1.0 compared to ISO/DIS 20122 method. The improved ISO method was tested to saponify the unprocessed meat. Nevertheless, when performing the MASE using 2M KOH EtOH:H_2O (1:1;v:v) at 60°C for 30 min the saponification of meat was incomplete, making it impossible to obtain a clear hexane phase, even after several washing steps. Consequently, the reaction conditions were changed to the ones used in Albendea et al. (2024) (120°C for 20 min).

The two methods (now differing only in the solvents used in the KOH solution) were compared primarily in terms of ISs ratio and recoveries. For the evaluation of the ISs ratio, the analyses were performed only in 1D mode, as it provided enough information for this evaluation, saving time and money. As aforementioned, an uneven ISs distribution can lead to discrepancies in the results depending on which IS is used to quantify the MOH contamination (TBB or 2-MN). Additionally, lower recovery of one IS after saponification compared to others may indicate that MOH recovery could also depend on its composition. The ratio of TBB and 2-MN was compared on three different samples of unprocessed meat (bacon, pig rib, and beef rib) as shown in **Fig. 1**, together with the tolerance levels accepted by the JRC guidance (1.15) (Bratinova et al., 2023) and by the ISO-20122/2024 (1.25) (ISO/DIS 20122, 2024). The TBB/2-MN ratio obtained was consistent across the three types of meat tested for each MASE method. The closest results to the theoretical ratio (1.00) were observed when the 2M KOH solution in EtOH/H2O (1:1;v:v) was used (on average 1.08 ± 0.02),

which was largely within the tolerance limits set by both the JRC guidance (1.15) and ISO-20122/2024 (1.25). In contrast, when the saturated KOH solution in MeOH was used, the ratio (on average 1.24 ± 0.02) exceeded the JRC guidance threshold and was close or equal to the limit accepted by ISO-20122/2024 (**Fig 1**).

<Insert Figure 1>

Since the evaluation of ISs distribution revealed a different behavior between TBB and 2-MN when the saturated KOH solution in MeOH was used, it can be expected that MOAH recovery might also be affected. A recovery test was performed using a motor oil solution (Sternel) to spike the meat sample with the lowest MOH contamination (beef rib, containing 0.49 mg/kg of total MOH) at two different levels. At the lowest level, MOSH and MOAH were added at 1.4 and 1.8 mg/kg, respectively, and at the highest level at 3.2 and 4 mg/kg, respectively.

The recovery test was carried out using the LC-(GC×GC)-FID system in both 1D and 2D GC modes and the recovery results for each level of added MOSH and MOAH are shown in **Fig. 2**.

<Insert Figure 2>

As it can be observed in **Fig 2.A**, MOSH recovery (obtained using Cycy for quantification) was not impacted by the KOH solution used for the saponification and the results obtained after GC×GC analysis were similar to the ones obtained by GC. In all cases MOSH recoveries were in the range accepted by the JRC

guidance (Bratinova et al., 2023) (**Fig 2.A**), showing values from 90 ± 3 % to 98 ± 4 %. When the 2M KOH solution in EtOH:H_2O (1:1; v:v) was used, MOAH recoveries were similar to the ones found for MOSH, regardless of the IS used for the quantification (TBB or 2-MN), which was expected due to their similar extraction (**Fig 1**) . However, when the saturated MeOH KOH solution was used, the results obtained for MOAH recoveries were highly impacted by the IS selected for the quantification. When the quantification was performed with TBB, the MOAH recoveries decreased, resulting in values lower than the one accepted by the JRC guidance (Bratinova et al., 2023) (**Fig 2.B**). These differences between the MOAH recoveries obtained using TBB and 2-MN (ratio between recoveries on average 1.22) were equivalent to the ratio observed between the two ISs (on average 1.24), suggesting that the behavior of the MOAH present in Sternel was better represented by the 2-MN than by the TBB. Similar to MOSH recoveries, the results obtained for MOAH after GC×GC analysis were aligned with the ones found by GC.

Both methods provided consistent results for MOSH; however, the purpose of this work was not only to study the different subclasses of MOSH present in meat but also to evaluate overall MOH contamination and identify potential sources of contamination. Thus, MASE performed with a 2M KOH solution in EtOH:H_2O (1:1, v/v) was chosen as the optimal sample treatment. This enhanced that the use of 2M KOH solutions in EtOH:H_2O (1:1,v:v) is not only adequate for the saponification of edible oils, as previously demonstrated by Bauwens & Purcaro

(2024), but also for more complex matrices such as meat, providing the use of a higher temperature, i.e., 120°C.

The limit of quantification (LOQ) was calculated from the calibration curve as 10 times the standard deviation of 10 procedural blanks as requested in the JRC Guidance (Bratinova et al., 2023). Two calibration curves, in 1D and 2D modes, were built. Very similar coefficients of determination were obtained (i.e., $R^2 \geq 0.998$), but the 2D method showed a sensitivity 10 times higher by comparing the two slopes (See **Fig.S.1** in supplementary materials), with an estimated instrumental LOQ 3 times lower in 2D compared to 1D. However, the calculated LOQ from the procedural blank was very similar, as it was mainly impacted by the contribution and variability related to the procedural blank. The total blank corresponded to 0.15±0.04 mg/kg in 1D and 0.09±0.03 mg/kg in 2D. The value is slightly lower in the 2D analysis as some of the interferences coming from the blank are separated in the 2D space, thus not interfering anymore with the MOSH contribution (which are the main compounds present in the blank). This is even more evident if we look at the area of elution of POSH and MOAH. Anyway, extrapolating the concentration from the calibration curve of 10-times the standard deviation, values of 0.30 and 0.28 mg/kg were obtained in 1D and 2D, respectively. The LOQ was rounded to 0.3 mg/kg of unprocessed meat in both cases, being below the most restrictive LOQ required in the case of low-fat food, i.e., <4% of fat, which is 0.5 mg/kg (Bratinova et al., 2023). The LOQ of 0.30 mg/kg was generalized to all the classes of MOH, but values between LOD and LOQ were retained and reported in *italics* or marked with * for further discussion.

3.2. Occurrence of Mineral oil hydrocarbons in meat

In terms of MOH contamination, animal fat was found to be the second greatest contributor to MOSH chronic exposure among all the different food groups for infants and the elderly by the EFSA in 2012 (EFSA, 2012), which highlights the impact that animal products can have on human exposure to MOH. However, as has already been pointed out in the introduction, the information related to the MOH contamination present in commonly consumed unprocessed meat is highly limited, which makes this food group an unknown factor for the evaluation of human exposure to these contaminants. As this study was partially performed to cover that knowledge gap, different types of commonly consumed meat coming from various animals (pig, beef, chicken, and turkey) were targeted. Besides the evaluation of the MOH contamination present in meat in terms of total MOSH and MOAH and the carbon chain length, the elucidation of MOSH and MOAH subclasses was investigated, as requested by the EFSA (EFSA, 2023). The ultimate goal is not a toxicological evaluation but rather an evaluation of the possible impact of bioaccumulation and biomagnification through the trophic chain. Thus, the profile of the samples studied herein were compared with the only data available on MOSH accumulation.

3.2.1. Total content of MOSH and profile

MOH can be absorbed in the digestive system (e.g. for mammals, mainly in the small intestine) and can be distributed along the organism through the portal and/or the lymphatic system, always through passive processes (Miller et al., 1996). The specific mechanisms involved in the distribution and deposition of

426 MOH through various tissues have not been elucidated yet and may depend on
427 the molecular structure of MOH. So far, it has been shown that the MOAH
428 absorbed by animals can be extensively metabolized and excreted (Barp et al.,
429 2017; Carrillo et al., 2022b; Chuberre et al., 2019), whereas certain types of
430 MOSH can present a tendency to accumulate in different animal tissues (Barp et
431 al., 2014, 2017; EFSA, 2023). Thus, if no exogenous contamination sources
432 occur during meat handling, MOAH should not be present, while MOSH can
433 derive from the accumulation due to the exposition of the animal to different MOH
434 sources. This is the case for most of the meat samples analyzed in this study
435 (**Fig. 3**).

436

437 **<Insert Figure 3>**

438 MOSH contamination ranged between <LOQ and 58 mg/kg, with an overall
439 average of 4.3 mg/kg and a median of 0.9 mg/kg. Only two samples (i.e., beef rib
440 3 and 5) showed very high levels of MOSH, namely 18 and 58 mg/kg, associated
441 with MOAH contamination as well (3.2 and 11 mg/kg, respectively). Only 7 out of
442 30 meat samples presented non-quantifiable levels of MOSH (4 pig ribs, 2 turkey
443 and 1 chicken samples).

444 As MOSH are liposoluble contaminants, higher concentrations might be expected
445 in meat with higher fat content. Turkey was the group with the lowest total lipid
446 content (on average 1.4%, see **Table S.1** in supplementary materials), but it had
447 a general MOSH level similar to pig rib (**Fig.3**), which had an average fat content

of ~23% (**Table S.1** in supplementary materials). These MOSH levels were similar to the ones observed by Albendea et al. (2024) in loin tissues (0.54 – 1.02 mg/kg of MOSH) with also a low fat content (estimated in 1%). The highest concentrations of MOSH were found in beef rib (**Fig 3**), particularly beef rib 5 showed MOSH levels (57 mg/kg, 18% fat content) almost twice the highest contamination found in pig's back fat (10 – 30 mg/kg, ~80% fat) in Albendea et al. (2024). The second highest level of MOSH contamination found for beef rib 3 (16 mg/kg, fat content 15.8%) was within the range of levels reported in pig's back fat in that study (Albendea et al., 2024) and similar to the maximum MOSH levels observed in beef rump steak (16.8 mg/kg) in Van Heyst et al. (2018).

Regarding the MOSH profile according to the carbon chain length, a schematic comparison of the general profiles observed for the different types of meat is shown in **Fig 4A** (see **Table S.1** in supplementary materials for more detailed information). Meat samples with a different profile than the general one (beef rib 3, 5 and chicken 3 and 4) will be discussed independently later in this section. To evaluate whether the MOSH accumulation by the animal can be considered as a critical biomagnification for human exposure, a representation of the information available in the literature on MOSH accumulation range in different species and tissues (Albendea et al., 2024; Barp et al., 2014, 2017) was schematized in **Fig 4B and C**.

<Insert Figure 4>

As represented in **Fig 4**, the MOSH profile shows only one hump for most of the samples studied, but with a variable carbon chain lengths range, from starting points of *n*-C_{18}-C_{20} to ending points of *n*-C_{30}-C_{40}. Thus, for most of the meat samples, we can deduce a single source of contamination, which might be related to the accumulation of MOSH by the animal. For instance, the MOSH accumulation range in fat from rats was in the *n*-C_{13-31} range, centered on *n*-C_{18} (Barp et al., 2017), while in human fat was reported in the *n*-C_{16-36} range, centered on *n*-C_{23} (Barp et al., 2014). In this study, the general profiles aligned with the one observed in human fat (Barp et al., 2014), but the humps were centered on higher carbon chain lengths (between *n*-C_{26}-C_{30}). This shift towards heavier molecular weights in comparison to the accumulation observed in human fat was also reported in pigs (loin and back fat), with a MOSH hump between *n*-C_{20-40} and a maximum on *n*-C_{30} (Albendea et al., 2024). The MOSH profile of some samples in this study and in the one performed by Albendea et al. (2024) (**Fig 4A**) included compounds with a higher carbon chain length than the ones observed in adipose tissues of rats and humans, but they were present in the liver (**Fig 4B** and **4C**), indicating that they can be absorbed and retained in animal and human tissues.

Similar to the outcomes obtained in pigs' loin and back fat (Albendea et al., 2024), neither of the meat samples studied in this work showed MOSH lighter than *n*-C_{18}. Even if the animals could have been exposed to these light MOSH, their absence in meat can be justified by the easy exhalation of MOSH below *n*-C_{20} and the ability of *n*-C_{16} and *n*-C_{18} alkanes to be oxidized in the intestinal mucosa

of different animal species (Barp et al., 2014, 2017; Phillips et al., 2000; Pirow et al., 2019).

Among the meat samples studied, beef rib 3 and 5 stand out not only for showing higher MOSH levels than the rest of the meat samples but also for the profile of the contamination, which included MOSH of high carbon chain length. A profile comparison between these two beef rib samples is represented in **Fig 5A** and **5B**, along with a sample of chicken that showed a completely different profile (**Fig. 5C**).

<Insert Figure 5>

The presence of two humps in beef rib 5 (**Fig 5.B**) revealed two different contamination sources in this sample, whereas only one source of contamination could be hypothesized in beef rib 3 (**Fig 5.A**). The small first hump observed in beef rib 5 (*n*-C_{16-29}, centered on *n*-C_{24}) falls in the MOSH range of accumulation observed by Barp et al. (2014) in human fat (*n*-C_{16-36}, centered on *n*-C_{23}), which indicates that the presence of this first hump can be due to a possible accumulation of these MOSH by the animal. Regarding the heavier MOSH, the information currently available in the literature supports the poor absorption of alkanes and MOSH above *n*-C_{35} in humans (Barp et al., 2014, 2017; Pirow et al., 2019), whereas in pigs, MOSH higher than *n*-C_{40} were not highly deposited in the back fat, even if they were present in the animal diets (Albendea et al., 2024). Therefore, the second MOSH hump found in beef rib 5 (C_{29-50}, centered on C_{45}), as well as the contamination found in beef rib 3 (C_{25-50}, centered on C_{35}), might

correspond to the contamination that occurred during the slaughter or the manipulation of the meat (postmortem). The presence of hopanes (**Fig 5A** and **B**) aligned with this hypothesis. Even if hopanes were observed in human milk by Populin et al. (2004), they were absent in different tissues from humans (Biedermann et al., 2015) and from rats that were fed diets with hopanes (Barp et al., 2017), suggesting a low tendency of these compounds of being retained in adipose tissues or intramuscular fat.

Among possible sources of contamination that could explain this MOSH profile found in beef rib 3 and 5, the possible transfer of these contaminants from food contact materials to the meat must be considered. Thus, an evaluation of the MOH contamination for the packaging of beef rib 3 and 5 was performed applying the method described in Di Mario et al. (2023), and the chromatograms obtained are shown in Fig 5.D and E, respectively. In terms of MOSH levels, the packaging of beef rib 5 showed a higher contamination (445 mg/kg) than the packaging of beef rib 3 (79 mg/kg), which agreed with the outcomes observed in the meat. Regarding the profiles, the chromatograms obtained (Fig 5) revealed that part of the MOSH contamination found in the meat could come from the packaging due to the similarity of MOSH profiles. However, the absence of hopanes in both packaging suggested that, in both cases, the contamination was not solely from the packaging. In the case of beef rib 5, the first hump observed in the meat was not present in the packaging, which supports the hypothesis that it could be due to the accumulation of MOSH in animal tissues.

A different MOSH profile from the general one (**Fig 4A**) was also observed in chicken 3 and 4. In these meat samples the contamination was characterized by two MOSH humps (**Fig 5C**) in the range of MOSH that was reported to be accumulated in the loin and back fat of pigs (Albendea et al., 2024). Thus, the entire MOSH contamination of these chicken samples might be due to the accumulation of MOSH by the animal, which implies that two sources of animal exposure to MOSH were revealed in the final meat intended for human consumption.

3.2.2. Subclasses of MOSH

The use of a reverse phase configuration (polar × non-polar) allowed an effective separation of the *n*- and iso- alkanes from the cyclo-alkanes and, therefore, to perform a more detailed profiling of the MOSH. Some of the *n*- alkanes present in food matrices have a natural origin and, therefore, they should not be considered as MOSH. According to the JRC guidance, these natural alkanes have primarily odd-number carbon atoms in their molecules, from n-C_{21} to n-C_{35} (Bratinova et al., 2023), which were observed only in some of the meat samples evaluated (in the range n-$C_{21\text{-}31}$) and considered as biological interferences. Following the JRC guidance, all the *n*- alkanes in the rest of the samples were considered MOSH. Nonetheless, it is important to notice that the metabolism of animals might alter this specific pattern of the natural *n*- alkanes observed in their diets, showing higher amounts of even *n*- alkanes than odd ones in their tissues (Tejeda et al., 2001).

A classification that allowed to differentiate MOSH into paraffines (*n*- + iso-alkanes), monocyclic and polycyclic was created using a motor oil and a technical white oil that contained all the MOSH subclasses (see **Fig S.2** in supplementary materials) and applied to the meat samples (**Fig 5**). Moreover, the presence of polyolefin oligomeric saturated hydrocarbons (POSH) was observed in most of the samples on top of the region of paraffines (*n*- and iso-) (see **Fig 4.C**), between *n*-C_{12} and *n*-$C_{24\text{-}30}$, having quantifiable levels only in one sample of turkey (**Fig.3**). After applying the classification for the estimation of MOSH subclasses, the relative percentage of each subclass was calculated for all the meat samples with quantifiable MOSH levels and represented in **Fig 6**.

<Insert Figure 6>

The relative percentage of the different MOSH subclasses was highly variable among the meat samples studied. No specific trend was found regarding a possible correlation between MOSH subclasses composition of the meat and the type of animal. The presence of MOSH in meat might be (entirely or partially) due to an accumulation of these contaminants in the animal tissue following the exposure of the animal, mainly through contaminated feed. However, the profile of the MOSH retained in animal tissues can differ from the original contamination present in the feed, as the accumulation depends on absorption, distribution through the organism, metabolization, and elimination. The information regarding the toxicokinetic of MOSH is limited, and it might vary depending on the animal species. Theoretically all types of MOSH can be metabolized in the small intestine and/or in the liver of mammals to the corresponding fatty alcohols through ω-

oxidation of the alkyl chain and then to fatty acids (EFSA, 2012). In the case of cycloalkanes, they can undergo ring oxidation, leading to the correspondent cyclanols, which can be subjected to ω-oxidation, whereas the branched alkanes can undergo ω1-oxidation of the branched chain (EFSA, 2012). The preference for one metabolic route over the others can lead to a change in the MOSH profile present in certain animal tissues in comparison to the original source of MOSH contamination. In the study performed by Biedermann et al. (2015), a preferential metabolic route of MOSH transformation was discarded by comparing the MOSH contamination in different human tissues with different possible sources of MOSH (i.e. batching oil, hydraulic oil, motor oil, and paraffin oil). According to this outcome, the variability of MOSH subclasses among the meat samples (**Fig. 6**) might be only due to animal exposure to different sources of MOSH, which led to a lack of correlation between the MOSH subclasses composition of the meat and the type of animal. Similarly, no relationship was found between the MOSH profile and the lipid content of the meat.

Considering the profiles of MOSH sources discussed in Biedermann et al. (2015), feed contaminated with a batching oil will contribute to a higher content of paraffines (concretely, *n*-alkanes and little branched) and monocyclic alkanes than polycyclic alkanes, which is the case of most of the meat samples (**Fig 6**). For instance, paraffines were present in all the meat samples and most of them (except chicken 6, turkey 1, beef 1 and 2, bacon 1 and 3, and pig rib 5) showed a proportion of this type of MOSH higher than the proportion of cycloalkanes, with the polycyclic being the minor subclass. On the other hand, Cravedi et al. (2017)

found an increase in open-chain hydrocarbons (*n*- and iso-alkanes) in adipose tissue of rats (58%) compared to the proportion in the MOSH mixture added to the feeds (≈ 40%), which would also support the paraffines being the main MOSH subclass in meat.

3.2.3. Total content of MOAH and subclasses

Only the two samples of beef rib that were highly contaminated with MOSH (numbers 3 and 5) showed quantifiable amounts of MOAH, shown in **Fig 3**. The MOAH absorbed by animals can be extensively metabolized and excreted (Barp et al., 2017; Carrillo et al., 2022b; Chuberre et al., 2019); therefore, the presence of MOAH in beef rib 3 and 5 supports a contamination of these meat samples during the slaughter or the manipulation of the meat for its commercialization. The presence of alkylbenzenes in the GC×GC chromatograms of both samples (**Fig. 7**) suggested that the contamination could be due to synthetic sulfonate surfactants probably used to clean machines intended to produce the different commercial cuts of the meat (Phillips et al., 2001; Venkatesan et al., 2002) or due to the migration from some offset-printed packaging (Aurela et al., 2001; Conchione et al., 2020). In this particular case, the possible contribution of the packaging to the MOAH contamination of the meat was ruled out, as the packaging of beef rib 3 and 5 showed no quantifiable levels of MOAH and the alkylbenzenes were absent in both cases.

The GC×GC chromatograms (**Fig. 7**) showed that the MOAH contamination was composed entirely of MOAH with highly alkylated one and two-rings.

Toxicological information available on the one and two-rings MOAH is not enough to raise a concern, however, it has been reported that some highly alkylated MOAH with one and two rings could act as tumor promoters in the mouse skin model (EFSA, 2012, 2019, 2023).

<Insert Figure 7>

4. Conclusions

The changes in the saponification solvent composition (EtOH:H_2O, 1:1; v:v) also proved efficient with meat samples to ensure a correct MOAH ISs ratio and, thus recovery. A minor adaptation of the previous method (120 °C rather than 60 °C) was necessary to ensure complete saponification.

The use of LC-GC×GC-FID/MS showed similar results to the ones obtained with LC-GC-FID, considered as the routine technique in MOH determination, giving at the same time more detailed information about the MOSH and MOAH contamination profile present in the unprocessed meat samples.

The MOH contamination was highly variable among the different meat samples, reflecting that highly different factors can affect the final contamination observed, such as the animal's exposure to MOH, the toxicokinetics of MOH in the different animals, or possible contamination sources during manipulation of the meat before its commercialization. No trend was observed regarding MOSH and total lipid content or the type of meat. MOAH were generally below the LOQ, except for two samples of beef rib containing significantly higher amounts of MOSH. In both cases, the MOAH were only 1-2 rings, with no traces of 3-7 rings.

Regarding the MOSH subclasses, in most of the meat samples, paraffines were present in a higher proportion than cyclo-alkanes, and polycyclic alkanes were the type of MOSH present in the lowest proportion. Further studies should be performed to understand possible preferential accumulation in different animal species by controlling the source of animal exposure to MOSH and assuring no other sources of MOSH contamination.

Based on the results of this study, meat consumption can contribute to human exposure to mostly paraffins.

Acknowledgements

The authors thank LECO, Restek, and Milestone for their support. This article is based upon work from the Sample Preparation Study Group and Network, supported by the Division of Analytical Chemistry of the European Chemical Society.

Funding

This work was partially supported by Fonds de la Recherche Scientifique Belgique (FNRS) (PDR projects-ToxAnaMOH T.0187.23).

CRediT authorship contribution statement

Paula Albendea: Data curation, Methodology, Validation, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis.

Giorgia Purcaro: Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

GP reports that financial support was provided by the Fund for Scientific Research. The authors declare that they have no other known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Albendea, P., Conchione, C., Menegoz Ursol, L., & Moret, S. (2024). A study on Mineral Oil Hydrocarbons (MOH) contamination in pig diets and its transfer to back fat and loin tissues. *Animals*, *14*(10), 1450. https://doi.org/10.3390/ani14101450

Aurela, B., Ohra-aho, T., & Söderhjelm, L. (2001). Migration of alkylbenzenes from packaging into food and Tenax. *Packaging Technology and Science*, *14*(2), 71–77. https://doi.org/10.1002/pts.534

Barp, L., Purcaro, G., Moret, S., & Conte, L. S. (2013). A high-sample-throughput LC-GC method for mineral oil determination. *Journal of Separation Science*, *36*(18), 3135–3139. https://doi.org/10.1002/jssc.201300114

Barp, L., Kornauth, C., Wuerger, T., Rudas, M., Biedermann, M., Reiner, A., Concin, N., & Grob, K. (2014). Mineral oil in human tissues, Part I: Concentrations and molecular mass distributions. *Food and Chemical Toxicology*, *72*, 312–321. https://doi.org/10.1016/j.fct.2014.04.029

Barp, L., Biedermann, M., Grob, K., Blas-Y-Estrada, F., Nygaard, U. C., Alexander, J., & Cravedi, J. P. (2017). Accumulation of mineral oil saturated hydrocarbons (MOSH) in female Fischer 344 rats: Comparison with human data and consequences for risk assessment. *Science of the Total Environment*, *575*, 1263–1278. https://doi.org/10.1016/j.scitotenv.2016.09.203

Bauwens, G., Gorska, A., & Purcaro, G. (2023a). The role of comprehensive two-dimensional gas chromatography in mineral oil determination. *Analytical and Bioanalytical Chemistry*, *415*(21), 5067–5082. https://doi.org/10.1007/s00216-023-04718-3

Bauwens, G., Barp, L., & Purcaro, G. (2023b). Validation of the liquid chromatography-comprehensive multidimensional gas chromatography-time-of-flight mass spectrometer/flame ionization detector platform for mineral oil analysis exploiting interlaboratory comparison data. *Green Analytical Chemistry*, *4*, 100047. https://doi.org/10.1016/j.greeac.2022.100047

Bauwens, G., & Purcaro, G. (2024). Improved microwave-assisted saponification to reduce the variability of MOAH determination in edible oils. *Analytica Chimica Acta*, *1312*, 342788. https://doi.org/10.1016/j.aca.2024.342788

Biedermann, M., Fiselier, K., & Grob, K. (2009). Aromatic hydrocarbons of mineral oil origin in foods: Method for determining the total concentration and first results. *Journal of Agricultural and Food Chemistry*, *57*(19), 8711–8721. https://doi.org/10.1021/jf901375e

Biedermann, M., & Grob, K. (2012). On-line coupled high performance liquid chromatography-gas chromatography for the analysis of contamination by mineral oil. Part 1: Method of analysis. *Journal of Chromatography A*, *1255*, 56–75. https://doi.org/10.1016/j.chroma.2012.05.095

721 Biedermann, M., Barp, L., Kornauth, C., Würger, T., Rudas, M., Reiner, A.,
722 Concin, N., & Grob, K. (2015). Mineral oil in human tissues, Part II:
723 Characterization of the accumulated hydrocarbons by comprehensive two-
724 dimensional gas chromatography. *Science of the Total Environment*, *506–*
725 *507*, 644–655. https://doi.org/10.1016/j.scitotenv.2014.07.038

726 Biedermann, M., Munoz, C., & Grob, K. (2017). Update of on-line coupled liquid
727 chromatography – gas chromatography for the analysis of mineral oil
728 hydrocarbons in foods and cosmetics. *Journal of Chromatography A*, *1521*,
729 140–149. https://doi.org/10.1016/j.chroma.2017.09.028

730 Bratinova, S., & Hoekstra, E. (2019). Guidance on sampling, analysis and data
731 reporting for the monitoring of mineral oil hydrocarbons in food and food
732 contact materials*. In EUR 29666 EN.* Luxembourg: Publications Office of
733 the European Union. https://doi.org/10.2760/208879

734 Bratinova, S., Robouch, P., Hoekstra, E., & Bratinova, S. (2023). Guidance on
735 sampling, analysis and data reporting for the monitoring of mineral oil
736 hydrocarbons in food and food contact materials In *EUR 31473 EN* (2nd
737 ed.). Luxembourg: Publications Office of the European Union.
738 https://doi.org/10.2760/963728

739 Carrillo, J.C., Shen, H., Momin, F. Kral, O., Schnieder, H., Kühn, S. (2022a)
740 GTL synthetic paraffin oil shows low liver and tissue retention compared to
741 mineral oil. *Food and Chemical Toxicology, 159,* 112701.
742 https://doi.org/10.1016/j.fct.2021.112701

743 Carrillo, J. C., Kamelia, L., Romanuka, J., Kral, O., Isola, A., Niemelä, H., &
744 Steneholm, A. (2022b). Comparison of PAC and MOAH for understanding
745 the carcinogenic and developmental toxicity potential of mineral oils.
746 *Regulatory Toxicology and Pharmacology*, *132*, 105193.
747 https://doi.org/10.1016/j.yrtph.2022.105193

748 Chuberre, B., Araviiskaia, E., Bieber, T., & Barbaud, A. (2019). Mineral oils and
749 waxes in cosmetics: an overview mainly based on the current European
750 regulations and the safety profile of these compounds. *Journal of the*
751 *European Academy of Dermatology and Venereology*, *33*(S7), 5–14.
752 https://doi.org/10.1111/jdv.15946

753 Conchione, C., Picon, C., Bortolomeazzi, R., & Moret, S. (2020). Hydrocarbon
754 contaminants in pizza boxes from the Italian market. *Food Packaging and*
755 *Shelf Life*, *25*, 100535. https://doi.org/10.1016/j.fpsl.2020.100535

756 Cravedi, J., Grob, K., Nygaard, U. C., & Alexander, J. (2017). Bioaccumulation
757 and toxicity of mineral oil hydrocarbons in rats - specificity of different
758 subclasses of a broad mixture relevant for human dietary exposures. *EFSA*
759 *Supporting Publications*, *14*(2), EN-1090.
760 https://doi.org/10.2903/sp.efsa.2017.en-1090

761 Di Mario, M., Bauwens, G., Peltier, F., Goscinny, S., Focant, J-F., Purcaro, G. &
762 Van Hoeck, E (2023), Investigation of potential migratables from paper and
763 board food contact materials. *Frontiers in Chemistry*, *11*, 322811. doi:
764 10.3389/fchem.2023.1322811

765 EFSA. (2012). Scientific Opinion on Mineral Oil Hydrocarbons in Food. *EFSA*
766 *Journal*, *10*(6), 2704. https://doi.org/10.2903/j.efsa.2012.2704

767 EFSA. (2019). Rapid risk assessment on the possible risk for public health due
768 to the contamination of infant formula and follow-on formula by mineral oil
769 aromatic hydrocarbons (MOAH). *EFSA Journal*, *16*(11).
770 https://doi.org/10.2903/sp.efsa.2019.en-1741

771 EFSA. (2023). Update of the risk assessment of mineral oil hydrocarbons in
772 food. *EFSA Journal*, *21*(9), 8215. https://doi.org/10.2903/j.efsa.2023.8215

773 ISO/DIS 20122. (2024). *Vegetable oils-Determination of mineral oil saturated*
774 *hydrocarbons (MOSH) and aromatic hydrocarbons (MOAH) with online*
775 *coupled HPLC-GC-FID analysis-Method for low limit of quantification*.
776 Retrieved from https://www.iso.org/obp/ui/en/#iso:std:iso:20122:dis:ed-
777 1:v1:en. Accesed November 30, 2024.

778 Isola, A. L., Carrillo, J. C., Lemaire, P., Niemelä, H., & Steneholm, A. (2023).
779 Lack of human-relevant adversity of MOSH retained in tissues: Analysis of
780 adversity and implications for regulatory assessment. *Regulatory*
781 *Toxicology and Pharmacology*, *137*, 105284.
782 https://doi.org/10.1016/j.yrtph.2022.105284

783 Menegoz Ursol, L., Conchione, C., Srbinovska, A., & Moret, S. (2022).
784 Optimization and validation of microwave assisted saponification (MAS)
785 followed by epoxidation for high-sensitivity determination of mineral oil
786 aromatic hydrocarbons (MOAH) in extra virgin olive oil. *Food Chemistry*,

370, 130966. https://doi.org/10.1016/j.foodchem.2021.130966

Miller, M. J., Lonardo, E. C., Greer, R. D., Bevan, C., Edwards, D. A., Smith, J. H., & Freeman, J. J. (1996). Variable responses of species and strains to white mineral oils and paraffin waxes. *Regulatory Toxicology and Pharmacology*, *23*(1), 55–68. https://doi.org/10.1006/rtph.1996.0009

Phillips, C. R., Venkatesan, M. I., & Lin, T. (2001). Linear alkylbenzenes in muscle tissues of white croaker near a large ocean outfall in Southern California, USA. *Environmental Toxicology and Chemistry*, *20*(2), 231–238. https://doi.org/10.1002/etc.5620200202

Phillips, M., Greenberg, J., & Cataneo, R. N. (2000). Effect of age on the profile of alkanes in normal human breath. *Free Radical Research*, *33*(1), 57–63. https://doi.org/10.1080/10715760000300611

Pirow, R., Blume, A., Hellwig, N., Herzler, M., Huhse, B., Hutzler, C., Pfaff, K., Thierse, H. J., Tralau, T., Vieth, B., & Luch, A. (2019). Mineral oil in food, cosmetic products, and in products regulated by other legislations. *Critical Reviews in Toxicology*, *49*(9), 742–789. https://doi.org/10.1080/10408444.2019.1694862

Populin, T., Biedermann, M., Grob, K., Moret, S., & Conte, L. (2004). Relative hopane content confirming the mineral origin of hydrocarbons contaminating foods and human milk. *Food Additives and Contaminants*, *21*(9), 893–904. https://doi.org/10.1080/02652030400001164

Tejeda, J. F., García, C., Petrón, M. J. J., Andrés, A. I. I., & Antequera, T. (2001). n-alkane content of intramuscular lipids of Iberian fresh ham from different feeding systems and crossbreeding. *Meat Science*, *57*(4), 371–377. https://doi.org/10.1016/S0309-1740(00)00114-5

Van Heyst, A., Vanlancker, M., Vercammen, J., Van den Houwe, K., Mertens, B., Elskens, M., & Van Hoeck, E. (2018). Analysis of mineral oil in food: results of a Belgian market survey. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *35*(10), 2062–2075. https://doi.org/10.1080/19440049.2018.1512758

Venkatesan, M. I., Northrup, T., & Phillips, C. R. (2002). Determination of linear alkylbenzenes in fish tissue by gel permeation chromatography and gas chromatography-mass spectrometry. *Journal of Chromatography A*, *942*(1–2), 223–230. https://doi.org/10.1016/S0021-9673(01)01400-5

Figure and Table Legends

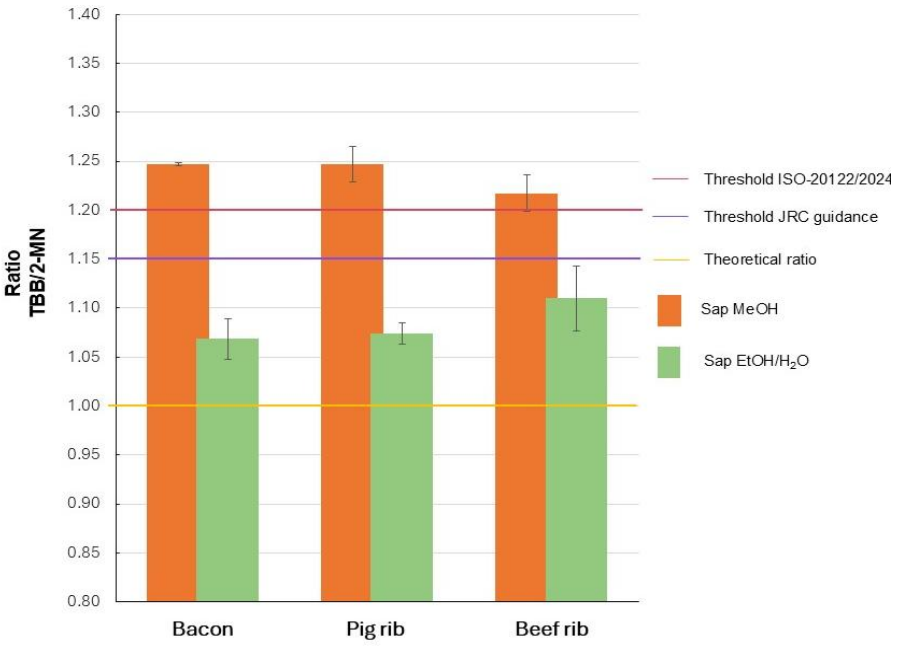


Fig. 1. *Comparison of the TBB/2-MN ratio obtained using two saponification methods: one with a saturated KOH solution in MeOH (abbreviated as Sap MeOH), and the other with a 2M KOH solution in an EtOH:H_2O mixture (1:1, v/v) (abbreviated as Sap EtOH:H_2O), applied to three different samples of unprocessed meat . The results are compared to the thresholds set by JRC guidance* (Bratinova et al., 2023) *and ISO-20122/2024* (ISO/DIS 20122, 2024)*. Data are presented as the mean and standard deviation of three replicates.*

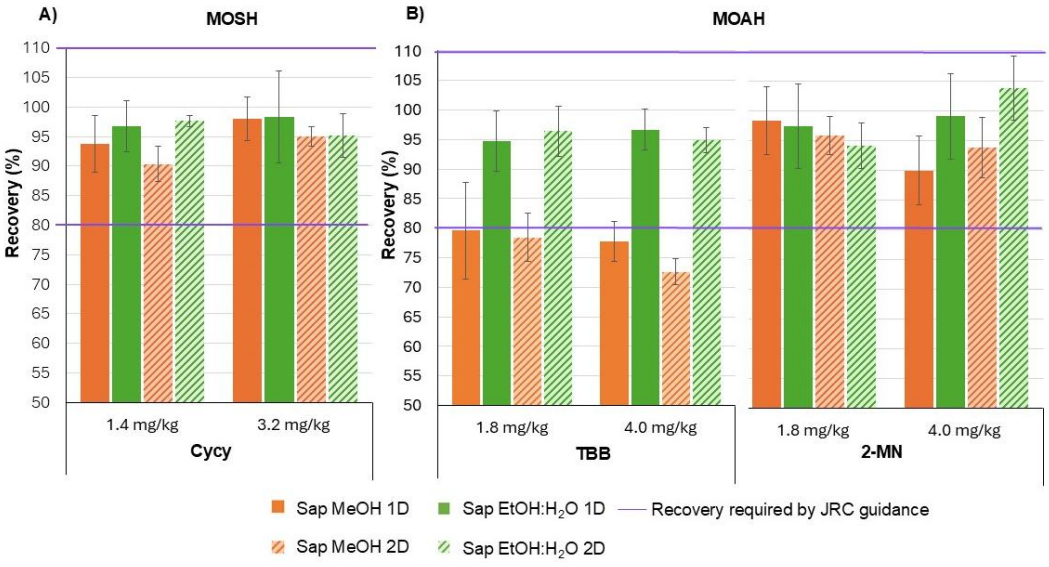


Fig. 2. *Recoveries obtained for MOSH (A) and MOAH (B) when a beef rib sample was spiked with two different levels of Sternel motor oil (1.4 mg/kg of MOSH plus 1.8 mg/kg of MOAH or 3.2 mg/kg of MOSH plus 4.0 mg/kg of MOAH), subjected to a saponification with a saturated KOH in MeOH solution (abbreviated as Sap MeOH) or with 2M KOH in EtOH:H2O (1:1; v:v) (abbreviated*

as Sap EtOH:H2O). Results were obtained by LC-(GC×GC)-FID in nondimensional mode (abbreviated as 1D) and bidimensional (abbreviated as 2D) and presented as the mean and the standard deviation of four replicates together with the recovery requirements specified by JRC guidance (Bratinova et al., 2023). For MOSH the quantification was performed with Cycy and for MOAH the calculations were made with TBB and 2-MN separately.

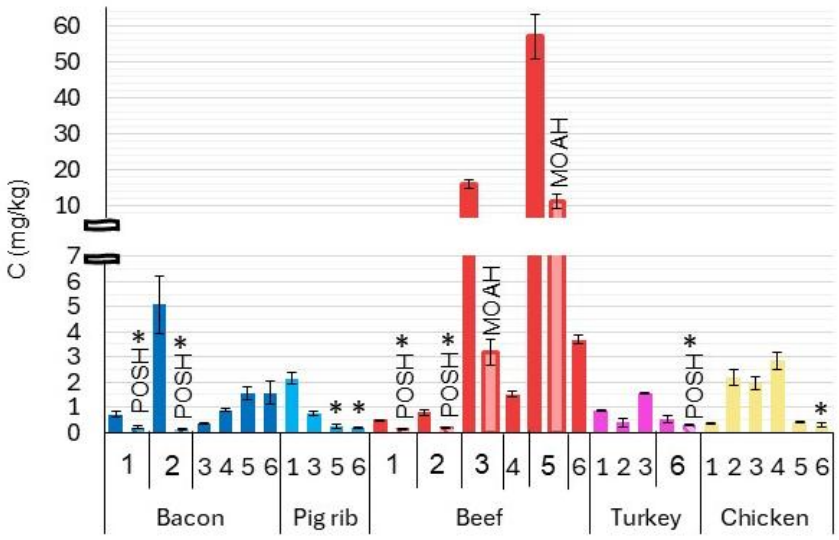


***Fig. 3.** Total MOSH levels obtained in the different meat samples studied (expressed as mg of MOSH per kg of meat). Only samples with detectable levels (>0.1 mg/kg) and quantifiable levels (>0.3 mg/kg) are included in this graph and highlighted. Detectable levels of POSH (polyolefin oligomeric saturated hydrocarbons) and MOAH, are also reported and labeled accordingly. (*)Values between LOD and LOQ.*

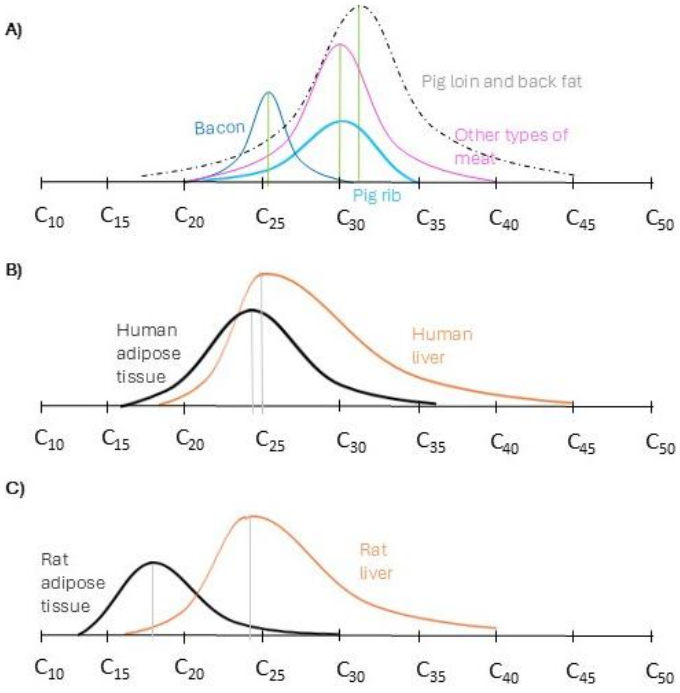
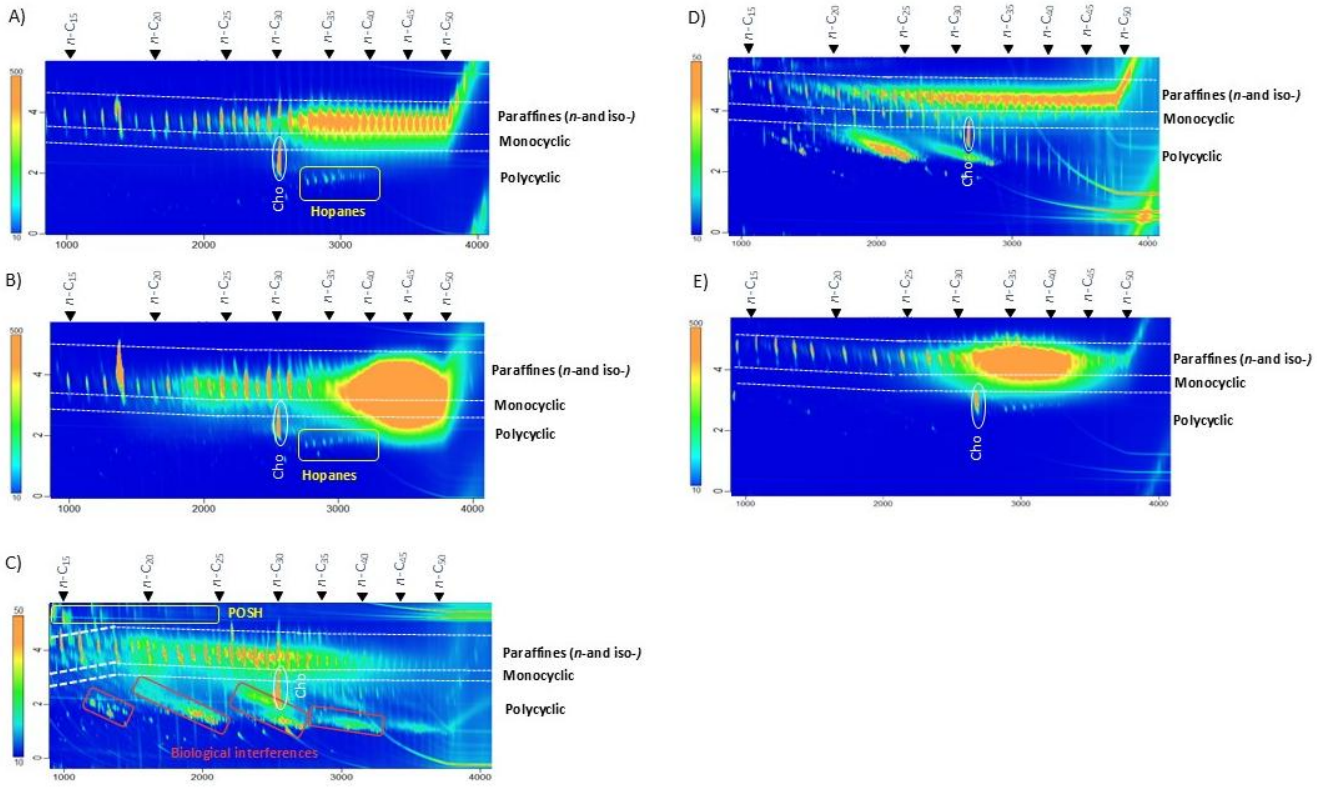


Fig. 4 *General representation of the MOSH profiles found in bacon, pig rib, the other types of meat evaluated in this study (beef rib, chicken and turkey) and in pig's loin and back fat by Albendea et al. (2024) (A); the MOSH profile observed in humans' adipose tissue and liver by Barp et al. (2014) (B); and the MOSH profile found F344 rats' adipose tissue and liver by Barp et al. (2017) (C).*



852

853 ***Fig. 5***. *Comparison of MOSH profiles observed in sample beef rib 3 (A), beef rib 5 (B), chicken 3*
854 *(C), packaging of beef rib 3 (D) and of beef rib 5 (E) after their analysis by LC-GC×GC-FID. The*
855 *position of different n-alkanes through the first dimension is marked by triangles on top of each*
856 *chromatogram and the internal standard cholestane (Cho) by white circles. The presence of*
857 *hopanes and POSH (polyolefin oligomeric saturated hydrocarbons) are marked with yellow*
858 *rectangles, whereas the presence of biological interferences is highlighted in red. The different*
859 *MOSH subclasses (i.e. paraffines, monocyclic and polycyclic compounds) are delimited by*
860 *discontinuous white lines.*

861

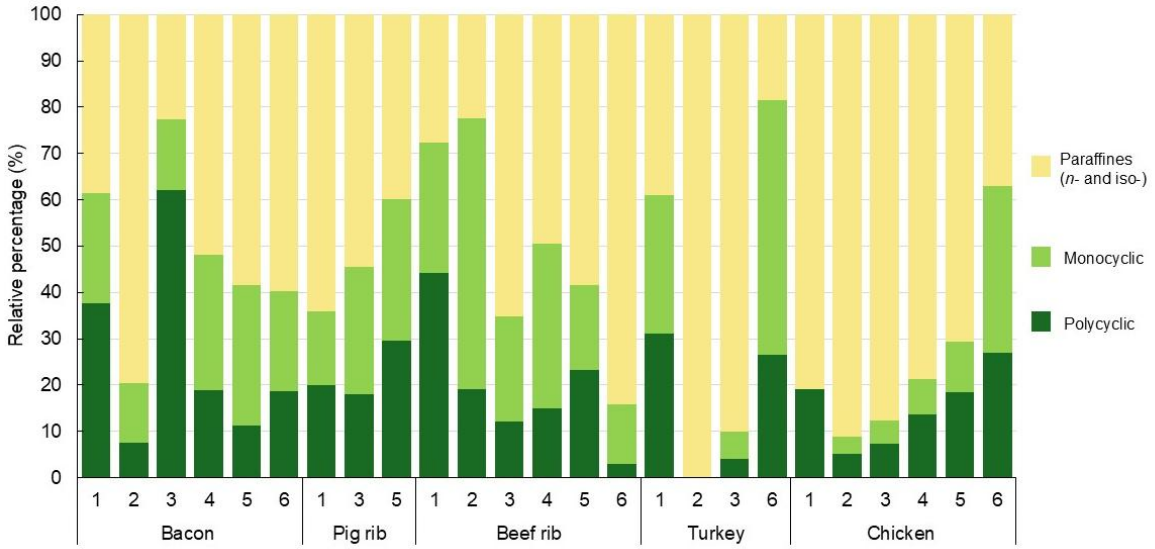


Fig. 6. *Relative percentage of MOSH subclasses (paraffines, monocyclic and polycyclic alkanes) with respect to the total MOSH levels found for the different meat samples studied with quantifiable MOSH levels.*

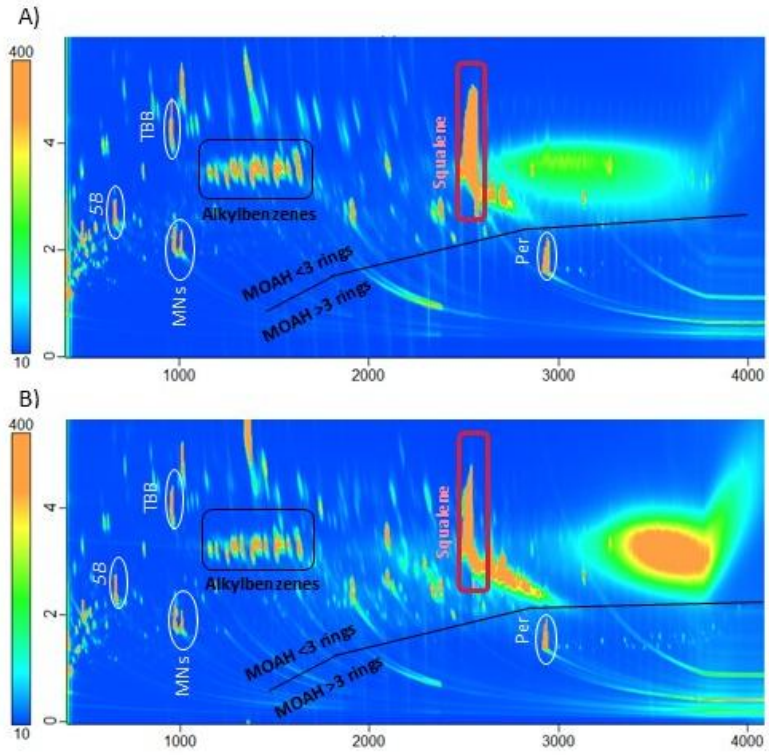


Fig. 7*. Comparison of the MOAH profile observed for the sample beef rib 3 (A) and beef rib 5 (B) after their analysis by LC-GC×GC-FID. The different internal standards are marked in withe, the different MOAH subclasses in black, and squalene (interference) is squared in red.*