Detection and Analysis of MOSH/MOAH Contamination in Edible Vegetable Oils: A Comprehensive Review

Kasturi Indiran[1a], Nur Hidayah Azeman[2b], Nadhratun Naiim Mobarak[3a*] and Ahmad Ashrif A. Bakar[4b]

Abstract: Consuming edible vegetable oil (EVO) is highly beneficial for human health due to its abundant nutrients, yet it might pose a risk if contaminated. Common pollution including mycotoxins, pesticides, heavy metals, and mineral oil are most likely to occur during harvesting, industrial processing, and environmental pathways. Particularly, mineral oil, a type of petrogenic pollutant can be further classified into two subgroups namely mineral oil saturated hydrocarbon (MOSH) and mineral oil aromatic hydrocarbon (MOAH). Exposure to MOSH/MOAH in EVO has a detrimental impact on human health, to the point that both subgroups are considered hazardous. Indeed, MOSH can bioaccumulate in specific organs, and certain MOAH compounds have a strong potential to cause cancer. The European Food Safety Association (EFSA) has established standards for vegetable oil, updated in 2022, which provide a maximum limit of 13 mg MOSH/kg and 0.5 mg MOAH/kg. Additionally, a pragmatic monitoring method is implemented, including identifying and evaluating MOSH/MOAH presence in the specific matrix using analytical techniques that can accurately measure their quantities. Despite the existence of various techniques, the Liquid Chromatography–Gas Chromatography-Flame Ionisation Detector (LC-GC-FID) technique stands out for its adaptability, efficiency, sensitivity, and excellent reproducibility, even in the presence of complicated matrix and analyte. Therefore, this study comprehensively explains the operating principle, sample preparation, column selection, and assessment results. Various advanced techniques including additional instruments; in certain instances, innovative approaches were employed to detect, measure, and distinguish individual MOSH/MOAH structures. Comprehensive analysis of the current literature, focusing on articles published between 2018 and 2024, is conducted to explore the occurrence and implications of MOSH/MOAH contamination in EVO. Additionally, this review delves into the application of the LC-GC-FID method, providing an in-depth evaluation of its efficacy in detecting and quantifying these contaminants within EVO matrices.

Keywords: MOSH/MOAH, edible vegetable oil, contamination, LC-GC-FID, chromatography.

1. Introduction

Mineral oil is derived from crude petroleum oil through refining processes, starting with atmospheric pressure distillation. Subsequently, the heaviest residues obtained from former process undergo vacuum distillation. The resulting filtrate, which has a boiling point ranging from 300 to 600°C, undergoes additional processing steps like solvent extraction, hyper treatment, and dewaxing, which are employed (Rawlings & Lombard 2012). At this stage, the resultant is referred to base oil, and further refinement yields mineral oil (MO) as finished goods (IARC 2012; Pirow et al. 2019).

Mineral oil can be defined as a mixture of various molecules with carbon numbers ranging from C1 to C50 (Rawlings & Lombard 2012). It is technically a blend of open or branched paraffinic compounds (saturated), naphthenic (cyclosaturated), and alkylated aromatic hydrocarbons (IARC 2012; Pirow et al. 2019; Weber et al. 2018). Hydrocarbon (HC) mixtures of the mentioned carbon count are relevant because compounds beyond this range pose high volatility and low absorption (Buijtenhuijs & Van de Ven 2019; Pirow et al. 2019).

Mineral oil (MO) can be structurally divided into two main categories: saturated hydrocarbons (MOSH) and aromatic hydrocarbons (MOAH), as illustrated in Figure 1. These terms were introduced by Biederman and his team in 2009 to distinguish mineral oil from pyrogenic polycyclic aromatic compounds (by-products of combustion) and hydrocarbons derived from plants (Pirow et al. 2019). The composition of both MOSH and MOAH can vary depending on the source of origin, which is why they are assigned different Chemical Abstract Service (CAS) numbers, as indicated in (Rawlings & Lombard 2012). Even when subjected to the same refining process, the association categorises them based on their description and characterisation (IARC 2012). Typically, MOSH constitutes the majority, ranging from 65% to 85%, compared to MOAH, which accounts for only about 15% to 35% of the total composition.

Authors information:

[a]Department of Chemical Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, MALAYSIA. E-mail: p126029@siswa.ukm.edu.my[1]; nadhratunnaiim@ukm.edu.my[3]
[b]Faculty of Enginnering and Build Environment, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, MALAYSIA. E-mail: nhidayah.az@ukm.edu.my[2]; ashrif@ukm.edu.my[4]
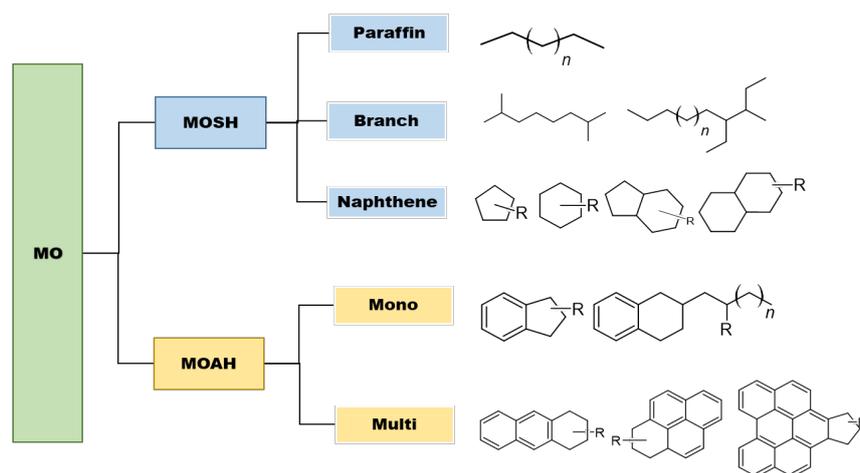*Corresponding Author: nadhratunnaiim@ukm.edu.my

Figure 1. Chemical structures of MOSH and MOAH (Weber et al. 2018)

Mineral Oil Saturated Hydrocarbon (MOSH)

MOSH is named after the characterisation by the chemical arrangement of linear or branched alkylated cycloalkanes such as paraffins or isoparaffins (e.g., n-undecane, bicyclohexyl, cyclohexyl cyclohexane) as well as naphthene (e.g., methylnaphthalene) (Srbinovska et al. 2020). In terms of terminology, MOSH is often referred by various names such as light mineral oil, white mineral oil, white oil, liquid paraffin, liquid petrolatum, mineral oil mist, paraffin oil, paraffinic liquid, petrolatum liquid, petroleum oil (Rawlings & Lombard 2012). This subgroup predominated over total MO, especially between a carbon number of C25- C35 depending on origin, while others were found in smaller fractions or below the limit of quantification (LOQ) (Srbinovska et al. 2020).

Mineral Oil Aromatic Hydrocarbon (MOAH)

MOAH comprises mono and/or polyaromatic hydrocarbons that are alkylated and hydrogenated. Despite the name aromatic, MOAH often has no scent, but in some cases, it is exceptional (Finch, Eilers & Harzke 2006). For instance, MOAH compounds are like perylene and 1,3,5-tri-tert-butylbenzene are fractions that can be separated from MOSH analytically (Jaén et al., 2022; Moret et al., 2016; Nestola, 2022; Rossum et al., 2022). Upon analysis, MOAH may result in a lower concentration, but it appears as a big hump that comprises a mix of aromatic hydrocarbon.

Thus, it is important to focus on petrogenic origin. In that mixture, polycyclic aromatic hydrocarbon (PAH) is usually mistaken for MOAH due to its inclusivity. However, it is a product of pyrolysis, where massive forest fires, volcanoes, and incomplete combustion of many sources are the reasons for its generation (Gharbi et al., 2016; Lachenmeier et al., 2017). Another nonpetrogenic HC, polyolefin oligomeric saturated hydrocarbon (POSH) contaminant found in edible oil where those compounds are in contact with or migrated from polyethylene (PE) or polypropylene (PP) packaging material (Gharbi et al. 2016; Srbinovska et al. 2020).

Properties of MO

Physical properties

MO is typically colourless (transparent), odourless, and tasteless in terms of their physical properties. However, there are exceptions to this general rule due to the complexity of the compounds (Rawlings & Lombard 2012; United States Pharmacopeial Convention 2014). In many cases, odorants are added to MO to provide a scent that warns of their presence for safety purposes or to mask any unpleasant odours (Finch, Eilers & Harzke 2006). This is supported by research indicating that MOSH and MOAH can potentially possess odorous characteristics, which can be detected individually or in a mixture through experiments conducted on mice (Gamble & Smith 2009).

MO exhibits variations in boiling points, which correspond to their specific applications (Finch, Eilers & Harzke 2006). The boiling point range typically falls between 218°C and 643°C. The flash point of MO is measured at 135°C for the closed cup method and 193°C for the open cup (OC) method. However, according to the ASTM D92 method (OC), a flash point of 115°C can also be triggered (United States Pharmacopeial Convention 2014). Generally, paraffinic oil tends to have higher flash and boiling points than naphthenic structures. Viscosity plays a crucial role in determining the molecular geometry, electrical, and thermal properties of the MO (Jin et al. 2014). Regarding kinematic viscosity, MO can exceed 38 mm²/s while maintaining its characteristic as a viscous liquid (United States Pharmacopeial Convention 2014; Yuliastuti 2010).

It is important to note that viscosity measurements should be accompanied by information about the test conditions, such as temperature, which is closely related to the pour point and influences the applicability of MO in specific uses. When analysing MO, the characterisation of properties like viscosity and the average molecular weight is often prioritised over determining the precise chemical composition, which can vary based on carbon number and structure (Polyakova, van Leeuwen & Peters 2022). Table 1 compares the general physio-chemical properties of EVO and MO, which makes sense for easy contamination.

Table 1. A comparison of physio-chemical properties between EVO and MO

Properties	EVO	MO
Origin	Plant	Petroleum (Animal Fossil)
Appearance	Light greenish yellow to red	Colourless
Viscosity	Varies; 33	>38
Density	Varies; 0.9	0.845 – 0.905
Solubility	Insoluble in water; Soluble in organic solvent, as in acetone, hexane and toluene	Insoluble in water & ethanol; Soluble in benzene, ether, carbon disulphide & volatile solvents
Pour point	-3ºC – 15ºC	~ -40ºC
Cloud point	-1ºC – 17.5ºC	< −8ºC
Fire point	323ºC – 378ºC	160ºC
Chemical stability	Oxidizable	Inert
Surface tension	31- 32 mN/m	40-60 mN/m

Additionally, MO has a partition coefficient in n-octanol/water of more than six (United States Pharmacopeial Convention 2014). The solubility of certain gaseous substances in MO has also been studied. According to Müller et al., (2012), nitrogen is highly soluble in MO compared to hydrogen and oxygen, which raises concerns for specific applications. Furthermore, a study by Liao et al., (2011) reported low moisture content in MO even after ageing, suggesting that moisture dissolves slowly in MO rather than absorbed by it.

Chemical Properties

Chemically, MO is mostly able to undergo a hydrogenation process, especially by naphthenic and aromatic compounds, leading to the formation of paraffinic and isoparaffinic HCs, which are widely used in the cosmetic and medical industries (Klaus, Tewksbury & Fenske 1962; Rausch et al. 1981). This chemical transition greatly impacts colour, odour, oxidation stability, and emulsification properties, not only by removing undesirable MOSH/MOAH but also nitrogen- and sulphur-containing compounds (Flinn et al. 1965; Wright n.d.).

Although MO is capable of acting as a strong oxidising agent and readily undergoes reduction under normal conditions, most MO compounds are chemically inert with respect to oxidation (United States Pharmacopeial Convention 2014). However, some compounds, particularly MOAH, tend to oxidise at elevated temperatures with excess oxygen to produce aldehyde, ketone and ester (Martin 2008; Zoccali et al. 2016).

This is like the EVO oxidation process, capable of rancidity over a prolonged period. The same phenomenon applies to the MO oxidation process, where its performance will be subpar due to sludge formation or crystallisation. To avoid this undesirable reaction, an oxidation inhibitor, such as sulphur, should be included in the formulation upon application to prolong the benefits (Kojic et al. 2019). MO has been used in various sectors, notably as a lubricant in machinery due to its high-temperature stability (Tuei 2023).

Thermal Properties

In addition to the properties above, MO also exhibits various thermal properties. The decomposition temperature of MO was initially unavailable, but it was predicted using time-temperature functionality to be around 371°C after 50 hours, as mentioned in Klaus et al., (1962). Later, the onset decomposition temperature was determined to be approximately 291.2°C using thermogravimetric analysis (TGA) by Patel et al., (2018). However, it should be noted that the actual decomposition temperature may vary depending on the specific application. For instance, in a research by Liao et al., (2011), a lower value was recorded for MO in an impregnated insulating pressboard system.

The thermal conductivity of MO, determined through the ASTM D2717 hot wire method, is an essential property for heat transfer under temperature differential conditions. In this test, a constant voltage is applied to a platinum wire, and the change in voltage flow is recorded as a function of the sample's thickness (Jin et al. 2014). The thermal conductivity value of MO has been reported as approximately 1.11 W/mK (Jin 2015). This property plays a crucial role in managing the heat generated by the magnetic circuit and windings in transformers, allowing for extended usability without causing harm to the circuit. Overall, the thermal properties of MO play a significant role in determining its shelf life.

Electrical Properties

As MO is a soup of all hydrocarbons, naphthenic compounds are highly valued for their versatile properties. They are extensively used in electrical appliances like insulating transformers due to their electrical characteristics, cost-effectiveness, and machinery efficiency (Rouabeh et al. 2019). Another essential electrical criterion is the dielectric constant (DC), also known as relative permittivity, which measures a material's ability to store electrical charge or energy relative to a vacuum. As a non-conductive material, MO exhibits a low DC value of approximately 2.22 at 90°C, which depends on its composition (Spohner 2017).

The DC value is influenced by internal factors such as polarity and oxidation tendencies of the oil (Martin 2008; Toudja et al. 2014; Yuliastuti 2010). While a high DC value is undesirable as it can lead to failure under intense electric fields, even a low value is sufficient for energy storage and other withstand capabilities. MO's suitability for transformer oil insulation, particularly in high-voltage electrical units, is attributed to its low DC value and ability

to keep the unit cool by preventing heat transfer during its shelf life (Jin et al. 2014).

Another important characteristic is the dissipation factor (DF), which represents the inefficiency of an insulating material and is defined as the tangent of the loss angle between changes in applied voltage and resulting current. A lower dissipation factor indicates better insulation. Tests conducted according to IEC 60247 standards compared the DF of MO with synthetic ester oil under different voltage conditions (Yuliastuti 2010). Generally, a good insulating oil exhibits a low DF, although it may increase with rising temperatures and conductivity (Rouabeh et al. 2019).

Next, breakdown voltage (BDV), the insulation strength, is measured where the electric field generates enough energy to ionise the electrons, and this causes chemical reactions that affect the performance of the dielectric properties. This is supported by Suwarno and Darma, (2008), who mentioned that MOAH is oxidised and forms precipitation in the form of sludge, which eventually reduces the initial BDV value. The BDV of MO recorded in (Yuliastuti 2010) is 38.63 kV, and this value can vary under the influence of methyl ester, moisture content, and temperature, as measured and tabulated in Table 2.

Table 2. Brief comparison on electrical properties (Rouabeh et al. 2019; Spohner 2017)

Characteristics	MO	EVO (Olive oil)	Unit
Viscosity @40°C	9.3	175.5	cSt
Dielectric Constant	2.43 – 2.29	3.43 – 3.02*	-
Dissipation Factor	14e-4	11e-4	Tan δ
Breakdown Voltage	32	46.5	kV
Resistivity@ 40°C	5.7	3.1	TΩ/cm
Humidity	90	200	ppm
Electrical Conductivity @ 40°C	175e-3	322e-3	S/m
Electrical regency	128	175.5	kV/mm

*sunflower oil

MO Application in the Food Industry

The versatile properties of MO make it highly valuable in numerous industrial and consumer applications. However, this review primarily focuses on MOSH/MOAH in EVO, with only brief discussions regarding food applications. In the food industry, food-grade MO is used as additives, glazing agents for candy, sprays for baked goods, and surface-treating agents for fruits and vegetables. Additionally, highly refined and treated MO is employed in the animal feed production (Buijtenhuijs & Van de Ven 2019; Moret et al. 2016). MOSH-derived paraffinic and naphthenic waxes find application in crop protection products, extending the shelf life of agricultural goods. It is also worth noting that MO is commonly used as a lubricant in mechanical components like bearings and gears in food manufacturing machinery. Despite the diverse uses, it is crucial to acknowledge that exposure or ingestion of MO above certain threshold levels can severely impact human well-being and the ecosystem within our food supply chain.

Contamination of MO in EVO

MO exposure in EVO occurs due to intentional or unintentional contamination throughout the production process of the primary raw material, vegetable oil. In 2008, a significant concern arose when sunflower oil exported from Ukraine to European countries contained MO levels exceeding 1000 mg/kg (Ahmad et al. 2019; Nestola 2022). The contamination was suspected of fraud or intentional admixture of base oil used in lubricating oil production rather than migration (Biedermann & Grob 2009).

However, according to Wrona et al., (2013), the typical contamination of MO in food is 10-100 μg/g, with additional amounts contributed by migration from packaging. Migration predominantly occurs through the gas phase and direct contact, with the gas phase being susceptible to MOSH in the range of C16 to C35 and MOAH below C25, while direct contact depends on the origin of contamination and is not limited to saturated structures (Buijtenhuijs & Van de Ven 2019; Moret et al. 2016).

Figure 2 illustrates the general pathways of MOSH/MOAH contamination and migration into food products, including EVO, encompassing environmental sources such as the atmosphere and aquatic ecosystems (EFSA 2012). Air pollution studies conducted by Buijtenhuijs and Van de Ven, (2019), revealed MO concentrations ranging from 0.03 to 5 parts per billion (ppb) in rural areas, particularly near road tunnels, where contamination or deposition is common. This contamination extends to plantation areas, where crops are harvested under the influence of MOSH/MOAH content and proceed to the processing stage. During processing, MO contamination increases due to leakage from machinery, including engine oil, lubricating oil, heating oil, and diesel oil in mills that come into contact with edible oil due to inadequate machinery maintenance or improper Good Manufacturing Practices (GMP) (Jaén et al., 2022).
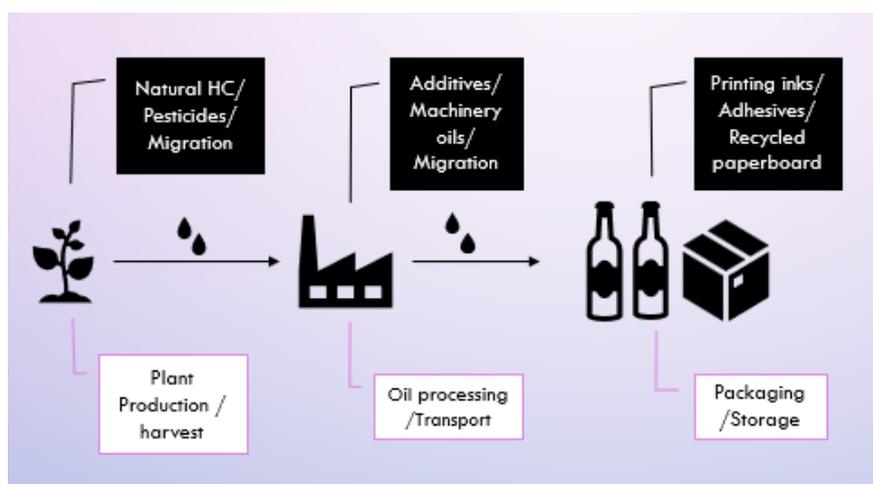
Figure 2. MOSH/MOAH contamination routes (adapted from Oppermann and Leitner, 2017)

Additionally, the use of recycled cardboard for packaging, printed with mineral oil-based inks, has been identified as a source of contamination. The printing inks and certain paper and packaging adhesives contribute to MOSH/MOAH contamination due to their sorption, diffusion properties, and partition coefficients (Fengler & Gruber 2022; Wrona, Pezo & Nerin 2013). According to the lab-scale experiment by Purcaro (n.d.), lighter C-fractions cause migration from jute bags to cocoa beans to occur more frequently due to its high volatility. The cocoa bean's shell functions as a barrier, letting only the lightest portion to get to the nibs. The total migration of pollutants depends critically on contact time. In some cases, migration of MO from adhesives is mistakenly attributed to contamination from the cardboard itself. It was found that one gram of hot glue used in food packaging can contain up to 8.2-118 mg of MOSH and 59 mg of MOAH in dry foods as shown in experiment by Lommatzsch and other fellows, (2016).

Various studies have confirmed the presence of MOSH/MOAH in food samples from 120 different countries, with nearly half of the samples (43%) testing positive for MOAH in 2015 (Jaén et al. 2021). Specifically, researcher Buijtenhuijs and Van de Ven (2019) investigated foods prepared with vegetable oil such as ice cream, desserts, and pasta and they found contamination levels of 0.4 mg/kg for MOSH and 0.028 mg/kg for MOAH in food product with a sample population of children aged 2-6 years. More shockingly, in 2019, the EFSA published an opinion on the presence of MOAH in infant formula, quantitatively assessing contamination levels ranging from 0.5 to 3 mg/kg (Hochegger et al. 2022). While highly refined MO is intentionally used in food products, non-intentional contamination can occur during various stages, including processing, packaging, transport, and storage (García-Cicourel et al. 2020). According to Pirow et al., (2019), the highest value obtained for vegetable oil is about 41-45mg/kg.

The mobility of MO compounds in edible oil depends on volatility, re-condensability, and vapour pressure, which determine the contamination rate or migration (Lorenzini et al. 2010). Precisely, medium-sized MO compounds with molecular mass up to C24 have high volatility, and the velocity depends on temperature, where high temperature can cause rapid migration with a wide range of hydrocarbon (HC) from contaminated packaging into the oil (Fengler & Gruber 2022).

Other factors that affect the rate include storage conditions, the structure of foods (high-fat content), which tend to have high migration, and the physiochemical properties of the barriers (Buijtenhuijs & Van de Ven 2019; Lorenzini et al. 2013). They recommend a temperature range between 20 to 40 °C and a 1 to 12 days storage time, indicating actual migration conditions. A migration test at equivalent conditions was done, whereby 60-80% of MOSH/MOAH compounds up to C24 proved to evaporate and migrate from paperboard into food by using modified polyphenylene oxide (MPPO – Tenax®) (Lorenzini et al. 2010).

Impact of MO towards human

Studies conducted on animals have investigated the effects of MO on tissues and illnesses associated with humans. Reference by Pirow et al., (2019) provides a summary of MOSH/MOAH levels found in animal and human tissues, as well as the observed effects such as increased organ weight, the presence of microgranulomas in mesenteric lymph nodes or liver, and changes in haematological and clinical chemistry parameters. These effects were observed in laboratory rats. In particular, rats' intake of MO in the form of transformer oil under designated conditions raised significant concerns, as it led to various toxicities affecting the skin, blood, liver, kidneys, and small intestines (Otunga et al. 2019).

MOSH can bioaccumulate in tissues, especially at lymph nodes, spleen and liver, leading to serious illness as mentioned above (Hochegger et al. 2021; Ruiz et al. 2021). If lymph nodes malfunction, it will lead to infections, blockage (build-up of body fluid) and cancer. This applies to almost all synthetic HCs like polyolefin oligomeric hydrocarbon (POH) and POSH. Although the human body is gifted with metabolism, only some low and medium-mass oil below C20 can be converted into smaller volatile compounds and excreted (Wrona, Pezo & Nerin 2013). In early 2024, a study found that pig's back fat showed distinct patterns of bioaccumulation, especially for MOSH with higher carbon numbers (n-C24 to n-C36, centered on n-C32) (Albendea et al. 2024).

Naphthenic hydrocarbon in the range of C20- C35, also called 'grey cloud' fractions, are likely to be retained and absorbed in the liver due to a lower elimination rate than iso and cycloalkanes, causing the formation of lipogranuloma (Carrillo et al. 2022). However, in late 2022, an assessment on MOSH adversity and relatability of animal and human tissue was discussed, noting that retention of HC of C20-C35, was not the cause of microgranuloma formation in rats (F344) and, therefore, not relevant to human conditions (Isola et al. 2023). Nevertheless, this scope of study is still debatable and requires detailed data for verification.

Meanwhile, certain MOAH compounds is surely recognised as potential carcinogenic and mutagenic substances, as mentioned earlier, where compounds with 3-7 ring PAC are considered so (Hochegger et al. 2022; Pirow et al. 2019; Ruiz et al. 2021; Srbinovska et al. 2020). These compounds are indicative of unrefined or poor process quality. In addition, short alkylated MOAH also contains heteroatoms that can induce cancer cell (Pirow et al. 2019). Not only that, a tumour may develop due to recurrence of dermal irritation at the stage where it cannot mutate or activate the cancer cell (Pirow et al. 2019). This is supported by (Hochegger et al. 2022), whereby selective MOAH compounds are involved in tumour development and DNA alteration via a non-genotoxic mechanism. MOAH exposure is, therefore, creating quite a concern among people, and the consumable product (food and cosmetics) should be free from MOAH or hazardous MOSH compounds.

2. Guideline

An extensive contamination of MO in sunflower oil makes the EU Commission established a limitation of 50 mg/kg for paraffin content in the sunflower oil (Gómez-Coca et al. 2016). Later, EFSA's Panel on Contamination in the Food Chain (CONTAM) issued a scientific opinion on the presence of MO in food based on a few case studies (Fiselier & Grob 2009; Pirow et al. 2019; Ruiz et al. 2021). In addition, preliminary data on the acceptable daily intake (ADI) of medium- and low-viscosity foods MO are published, although these data are from poor analysis (EFSA 2012). Based on this publication, the European Union issued a proposal (EU 2017/84) shortly after in 2017 to monitor MO, focusing on its content in food and food packaging (Andriukaitis 2017).

In the year 2022, the Member States of European countries agreed that MOAH concentration should be followed without regard to fractions in a variety of food products that focus on infant formula and toddler food, irrespective of the MOAH sources, as stated in Table 3 (European Commission 2022). An updated version of the scientific opinion was drafted in 2023 after some thorough toxicity evaluation. As per EFSA and fellow contributors (2023), most of the uncertainty was clarified, including conclusions of the EFSA (2012) opinion that the genotoxicity of MOH is associated with the presence of three or more ring MOAH.

Table 3. Benchmark for MOSH/MOAH in food related product

Food Categories	Limit value (mg/Kg)		References
	MOSH	MOAH	
Dry foods with a low-fat content (≤ 4% fat/oil	-	0.5	(European Commission 2022; Parkinson 2022)
Foods with a higher fat content (> 4% fat/oil, ≤ 50 % fat/oil)	-	1	
Fats/oils and foods with (> 50 % fat/oil)	-	2	
Vegetable oil	13	0.5	(Sabrina & Giorgia 2022)
Dry food: bread, pastry, cakes, biscuits, cereal	6	0.5	(Institute for Publication n.d.)
Confectionary except	9	0.5	
Migration from packaging	0.6	0.5	(Pirow et al. 2019)

In certain food categories, MOSH has no permissible limits for the migration that recognised as non-dangerous, while MOAH should be below 0.5 mg/Kg as the limit of determination (LOD), which cannot be exceeded analytically as stated in Table 3. However, the estimation of human exposure is less accurate, so that still no "tolerable daily intake" (TDI) has been registered by a national legislation (Buijtenhuijs & Van de Ven 2019). Additional toxicity data are needed, especially on bioaccumulation of MOSH and potential effects on human tissues, i.e., inflammation, liver and spleen diseases. Furthermore, the technical specifications for MOAH used as food additives and packaging materials should be updated to avoid composition and content of three or more ring compounds. The issue is difficult due to its complexity, both in terms of the toxicological evaluation (which requires characterizing the toxicity of each molecule and their interactions, such as synergism or antagonism) and the analytical determination (Matheson 2023).

3. Analysis

Detection Method

Initially, the gravimetric analysis (IP 346 method) was introduced by the European Petroleum Refiners Association specifically for testing unused lubricating oil to ensure that carcinogens are not detected as well as to maintain the quality of MO (Ruiz et al. 2021). There are three categories of petroleum: lubricating oil, foot oil, and treated distillate (aromatic extracts), the latter of, is considered a carcinogen under certain conditions according to CLP Regulation No. 1272/2008 (Pirow et al. 2019). To determine the presence of those compounds, this method has been utilised as a qualifier for refining efficacy. According to Hochegger et al., (2022), this method also measures and evaluates the weight percentage of PACs in dimethyl sulfoxide (DMSO) extracts of lubricating oil to obtain good quality.

In earlier times, infrared (IR) spectroscopy was used to detect MO through the C-H stretching vibration. Still, this method is the most ineffective as it is non-quantitative and cannot distinguish the source (EFSA 2012). Another test, AMES, is the common method for testing mutagenic propertied compounds using biological assays like bacteria. This method was named after its developer, Bruce N. Ames, Berkeley and is especially used in the pharmaceutical and cosmetic industries where compounds are formulated into (Boogaard et al. 2012). Compounds that fall under mutagenic criteria are usually considered carcinogenic, too. Therefore, this test was modified to detect such complexes using bacteria (Biopharma n.d.; Boogaard et al. 2012; Hochegger et al. 2022).

Next, Hochegger et al. (2022) revealed that manual separation into MOSH and MOAH using silica was followed by HPLC clean separation, during which MOAH was further separated into three-ring and larger (>3 ring) MOAH structures. HPLC is a vital instrument for investigation and quality control measures related to edible oils because of its ability to perform high-resolution separations and detect trace quantities of constituents (Ali & Neha 2024). Then the resultant further conditioned before proceeding with the AMES test. In brief, a 10µl sample was placed in duplicate on 24-well plates and diluted with a combination comprising 5% (v/v) bacteria (Salmonella typhimurium TA98, about 109 CFU/mL) and 4.5% (v/v) phenobarbital/-naphthoflavone-activated rat liver S9, which was incubated for 90 minutes at 37°C with agitation. The mixture was then dispersed into 48 wells with a reversion indicator and incubated for another 2-3 days. When revertant levels were 2-fold higher than the baseline, samples were identified as DNA reactive or carcinogens, as shown in Figure 3.
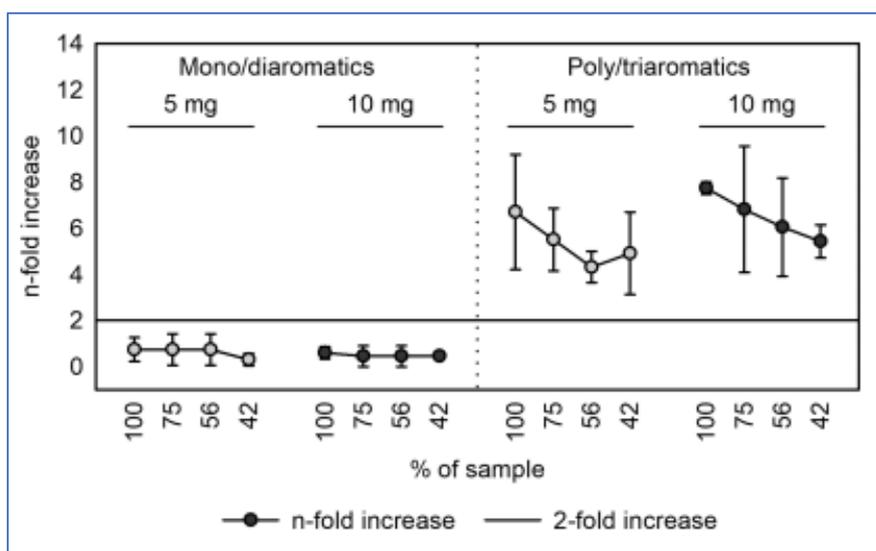

Figure 3. AMES test result for mono-diaromatic and polyaromatic fractionates with 250µl DMSO extraction (Hochegger et al. 2022)

In addition, the mouse skin painting model also serves as an indicator for toxicity assay, as human and mouse skin may develop similar dermatological effects (tumour) when exposed to the carcinogenic MO substance (Carrillo et al. 2019). This study provides the most realistic response to chemical consumption at various parameters such as latency, mechanism including multiplicity and malignancy, etc. (Walaszek, Hanausek & Slaga 2007). Another test using alcoholic potassium hydroxide solution with distilled water, where turbidity indicates concentration after slight mixing, is another traditional method for detecting MO in cooking oil.

Due to the massive contamination of MO in sunflower oil, 2008 IUPAC (1987) and AOCS (1997) were standardised for contamination in oil and fat, measured by densitometry and column chromatography, respectively. But quantification of these methods yields a high limit of detection (LOD), about 500 – 1000mg/kg. The following section will brief on the current analytical approach that enables MO quantification in EVO and other complementary matrices, as well as room for alternative emerging methods.

MO Analysis by LC-GC-FID and its Preface

Initially, the quality of MO was the focus; soon after scientific reports were published, detecting MOSH/MOAH in foods was of

keen interest. Thus, an advanced technique, Liquid chromatography (LC) - Gas chromatography (GC), was reborn and is very applicable in MO detection and quantification, particularly in separating MOSH and MOAH individually. High-Performance Liquid Chromatography (HPLC) with capillary Gas Chromatography (GC) technique was introduced by Grob et al. in 1984 for the fractionation of trace components in the mixture. Later, this method was coupled with a flame ionisation detector (FID) for MOSH/MOAH determination in food samples in 2009. Since then, analysis of MOSH/MOAH has been intensively studied (M. Biedermann et al., 2011; M. Biedermann et al., 2009; M. Biedermann & Grob, 2015; Hochegger et al., 2021).

Moreover, method with a chromatographic separation column LC coupled with GC-FID was developed by Gómez-Coca et al.,(2016). Currently, this LC-GC-FID method is the most common technique due to its high sensitivity towards analytes and less solvent usage, which indirectly improvises the quantification (Moret et al. 2016; Purcaro et al. 2013; Wagner & Oellig 2019). In other words, it is considered as the gold standard for MO analysis in the refinery (Weber et al. 2018). Also, it provides high resolution featuring fully automated that enables analysis of a complex sample with less error in terms of contamination and interference (Biedermann, Fiselier & Grob 2009). Due to these features, technical guidance of the European Commission, Joint Research Centre (JRC) used online LC-GC-FID as analytical method for determining MOSH/MOAH in food products and result verification by GC-GC-MS for current EU-wide monitoring (Foodwatch 2021). This LC-GC-FID method uses internal standards or markers to identify MOSH/MOAH fractions, mostly, to control the performance of the entire system.

LC-GC-FID: Principle

First, LC ensures the determination of MOSH/MOAH fraction by distinguishing into paraffin, naphthene and aromatic according to retention or elution time, which correlated to molecular mass that is quantifiable by GC-FID under the same chromatographic conditions as shown in Figure 4 (Buijtenhuijs & Van de Ven 2019). A significant step where LC executes the isolation of analytes (in this case, MOSH and MOAH) from potential interference like lipids, plant HCs (C23-C35) and wax esters in food samples (EFSA 2012). This step is usually done in the normal phase with bare silica and is always up for improvement (García-Cicourel et al. 2019).
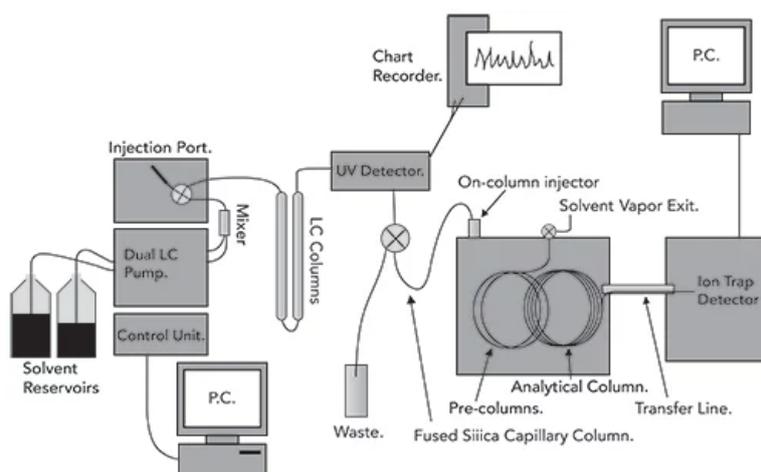

Figure 4. Schematic diagram of LC-GC-FID (Apedo & Snow 2019)

Then, the eluent is transferred to the GC column as described by Biedermann et al., (2009), using the retention gap technique with simultaneous eluent evaporation through the interface on the column. The interface must be carefully designed to handle the transition between these two phases. Techniques such as partially concurrent eluent evaporation (PCEE) are used to optimize this transfer, ensuring that volatile compounds are not lost during the process. The interface also minimizes issues like memory effects, which can occur when residues from previous samples affect the analysis of subsequent samples. This technique was well differentiated by Sdrigotti and team (2021) with fully concurrent eluent evaporation and solvent flooding.

Following to GC stage, the eluent is evaporated as per individual boiling point, and the gas molecules are injected into the stationary phase to determine a unique molecular mass. A nonpolar stationary phase with a short capillary column is commonly used, while the mobile phase uses inert gas like helium or nitrogen (EFSA 2012).

When the vaporised sample reaches FID, it is ionised in the flame by the reaction of hydrogen and oxygen, causing the ions to generate a current that is measured by the electrodes in the detector. This measurement will be displayed as a peak or signal at a certain time of elution in response to unit mass for HC. Therefore, this signal is directly proportional to the amount of targeted compound over a wide array of compounds (Weber et al. 2018). In addition, with this detection method, LOD for MOSH and MOAH is about 0.05mg/kg. But this is not the actual case for the oil sample, where paper by EFSA, (2012) & Fiorini et al., (2010) points out the LOD was recorded as 5 mg/kg with a modified GC-FID technique.

Although a FID can readily measure all HCs, it is not selective and has uncertain sensitivity regarding the formation of broad unresolved peaks of MO compounds (EFSA 2012). Alternatively, with the help of chemical markers and internal standards, this detection method can differentiate compounds in the chromatographic profile. This is done by identifying peaks, eliminating potential interference, and measuring quantification based on the total area (Ahmad et al. 2019; Biedermann, Uematsu & Grob 2011; Srbinovska et al. 2020). By having proper sample preparation (highly selective separation and elimination process) in the early stage, only then, target compounds (MOSH/MOAH) detected by this FID (Zoccali et al. 2016).

LC-GC-FID: Sample Preparation

Sample preparation is a critical step before the analytical run because it requires saponification (separation of triglycerides), epoxidation (separation of MOSH) and extraction (removal of olefins and endogenous n-alkanes) to achieve adequate sensitivity of determination (Hochegger et al. 2021; Srbinovska et al. 2020). Saponification was the pioneering step to separate from triglyceride, particularly in high-fat content samples (Hochegger et al. 2021, 2022). According to EFSA (2012), the saponification step is labour-, time-, and solvent-consuming and should therefore be performed meticulously to avoid additional errors in the analysis.

In the case of EVO as a sample matrix, epoxidation helps to separate MOSH from unsaponifiable matter to obtain more polar derivatives of unsaturated compounds that can be retained strongly in the LC (Gómez-Coca et al. 2016; Zoccali et al. 2016). However, conventional epoxidation is unable to detect MOAH as most of them are unaffected, thus need harsh or improved epoxidation to eliminate olefins. As per detailed explanation written in Nestola (2022), where the MOAH fraction can potentially co-elute with biogenic olefins, the researcher focused on the removal of biogenic interferences in palm oil samples through enhanced saponification and epoxidation in an automated online LC-GC-FID method, thus achieved a group of individual MOAH peaks (Albendea et al. 2024).

As per Sdrigotti et al. (2021), loss of 20-35% of MOAH may occur during these purification steps, as well as the removal of the interferences may remain incomplete. In terms of EVO, there are olefins, HCs (plant-based), and other trace compounds as interferences, that shall be removed through epoxidation in the occasion of off-line column chromatography used, the sample extraction stage proceeded (Ruiz et al. 2021). If not properly accounted for, co-migration of other HCs; POSH, native n-alkanes, terpenes and essential oils or sterol esters can cause an overestimation of MOSH/MOAH (Wagner & Oellig 2019). Particularly, natural polyolefins and unsaturated poly-a-olefins are misinterpreted for MOAH humps (Pirow et al. 2019).

In certain cases, hexane extraction is sufficient, like food products. However, complicated samples, such as those involving high-fat and cosmetic products, require an effective sample clean-up process (Weber et al. 2018). Lipid matrices require sophisticated decontamination techniques such as saponification and column chromatography using a silica or cartridge (Purcaro et al. 2013). Thus, choosing the optimal column for the best separation is considered significant.

LC-GC-FID: Separation Column

Since MOSH/MOAH is categorised into nonpolar compounds and is lipophilic, it is highly possible to dissolve well in nonpolar solvents and migrate to the lipids (Wrona, Pezo & Nerin 2013). Therefore, separating targeted chemical compounds from unwanted, may encounter some challenges. Few pre-treatments were carried out to avoid clumps of peaks and improve analyte identification sensitivity. Among that, solid phase extraction (SPE) has been widely used.

Regarding SPE, supercritical fluid chromatography has been utilised as a rapid and easy method to analyse MOSH/MOAH fractions in crude and refined oil, whereby detection is done by FID and UV detectors parallelly. However, this method of analysis is achievable if the oil viscosity ranges below 56 mm^2/s and the molecular weight is less than 450 g/mol. Additionally, extra precautions are taken to reduce matrix interferences to get more precise data (García-Cicourel et al. 2020). Likely, Gómez-Coca et al. (2016) used n-hexane as the mobile phase in a silica column loaded with silver (Ag) to separate HC fractions and interferences brought on by the high-affinity Ag+ ion for double bonds. As a result of limited solubility, bigger aliphatic structured compounds can retain more (García-Cicourel et al. 2020). For nonpolar fractions of EVO that were separated by silica gel-SPE step with n-hexane and detected by GC-MS similarly in Weber et al. (2018).

Additionally, activated silica gel impregnated with 1% silver nitrate ($AgNO_3$) acts as a sorbent in the column pack for offline chromatography. It separates MO from olefin and triglycerides (interference) by retaining them in the column (Ruiz et al. 2021). The elution sequence goes like low molecular weight paraffin to naphthene (MOSH fractions). It is followed by highly alkylated benzene and alkylated polyaromatic through a normal phase column (Weber et al. 2019).

Sulphuric acid-impregnated silica gel (SAISG) column was demonstrated by Wrona et al. (2013) for the extraction of edible oil at where it was capable of providing good quantification, unmistakable MO identification as well as allowing the removal of fatty acids and lipids without the creation of an emulsion. Additionally, Moret et al. (2016) suggested a more effective extraction technique called microwave-aided saponification (MAS), followed by online LC-GC with FID for the MO determination in various food products, particularly those with high fat content. A similar method of MAS was used by Srbinovska et al., (2020) to determine HC, including MOSH/MOAH in packed fish and fish products.

LC-GC-FID: Assessment

Basically, there are two: online LC-GC-FID and offline SPE-GC-FID techniques that have been widely used in MOSH/MOAH determination in EVO as a food matrix. Regarding online LC-GC-FID, saponification and extraction are first performed with n-hexane and water. In the former step, a few millilitres of oil samples are provisionally obtained and then fed to the epoxidation process before being injected into the LC-GC-FID system. At this time, the LC separation is performed according to the elution of MOSH at 2-4 minutes and MOAH at 4.1-6.1 minutes, then the sample is transferred to the GC by the retention gap technique or partial simultaneous solvent evaporation (PCSE) through the connector as described in Nestola (2022). At a specific

degree of alkylation, various structural isomers of MOAH can have similar volatility characteristics, leading to their elution within a narrow range of carbon atoms (up to 4 atoms) (Biedermann et al. 2022).

Moreover, parameters such as the inlet pressure of the carrier gas, the oven temperature, and the flow rate of the fraction were programmed before the run. Once the fraction exited the GC, data processing continued using FID to quantify each compound recorded in the peak/signal as shown in Figure 5. Cross-checking with GC-MS is recommended for molecular identification, as mentioned in the article by Nestola (2022).
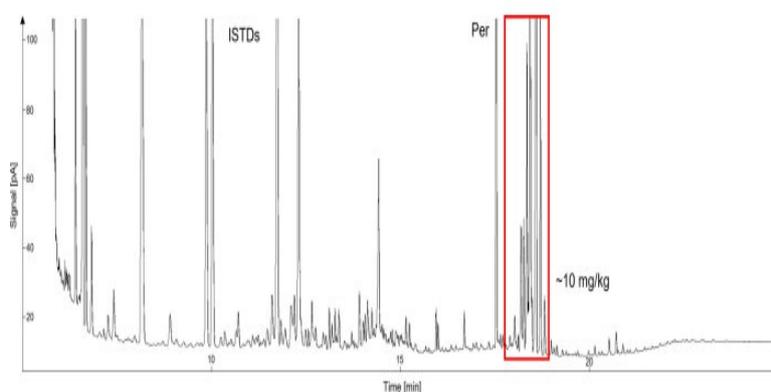

Figure 5. Chromatographic plot on MOAH by LC-GC-FID for EVO after epoxidation (Nestola 2022)

That being said, in the study published by Foodwatch (2021) also mentioned on GC*GC-TOF technology is efficient for results verification and marker assessment. Recently, GC×GC–TOF/MS has become a popular choice of analytical instrument because of its superior ability to separate complex mixtures of volatile compounds by distinguishing even subtle volatiles, offering better peak shape, enhanced purity, and greater accuracy in both qualitative and quantitative analyses. The chromatographic peaks are displayed on a 2D plane, giving each volatile two retention indices with a fixed position. Also, due to its super-fast spectral generation rate (up to 500 spectra per second), GC×GC–TOF/MS can analyze thousands of components in a very short period (Fang et al. 2023).

Back to offline SPE-GC-FID, this method is defined by the manual preparation of the chromatographic column, as the sorbent and activated silica gel column were compacted and followed by dropwise addition of AgNO$_3$ solution, then proceeded to vortex process to make it bubble-free and again to compact the sorbent together with mobile phase, n-hexane. This is the most important step for separating MOSH and MOAH after the epoxidation process, as described in Ruiz et al. (2021). The next step is the extraction, which was improvised to isolate interferences with solvents. In the work of J. C. Carrillo et al. (2019), DMSO was used as a solvent because it efficiently removes PAH and sulphur- and nitrogen-containing heterocycles.

This type of extraction was then developed as a combination with aliphatic solvents such as pentane and water to separate polycyclic compounds from aliphatic HCs and other organics. However, in some cases, a mixture of solvents such as toluene, n-hexane, and dichloromethane was used in a specific ratio to keep the sample from a complete evaporation (Ruiz et al. 2021). The extracted sample is then injected into the GC-FID, which has already been programmed for temperature and gas flow rate. The outcome of this detection of individual targeted compounds can be seen in signal or peak as a display. Especially on the aromatic compounds with high molecular weight, a specified hump is usually seen yet can be interpreted by the help of markers and internal standards (García-Cicourel et al. 2020; Gómez-Coca et al. 2016).

Method Performance

At the end of the plot, the performance of the analysis will be measured in terms of LOD, LOQ, linearity (R^2), and recovery. The detection limit depends on MO distribution in the sample amount that injected into the system but mostly, relies on the context of the oil or fat composition. To improve the detection, a higher capacity system and food origin markers (n-alkanes) are used (EFSA 2012). A complete guidance on sampling, analysis and data reporting was written in the frame of Commission Recommendation (EU) 2017/84 for MO determination (Hoekstra, Bratinova &

(Editors) 2019). The chosen method must meet certain performance parameters, including a response factor of 0.8-1.2 for C50 over C20, a target and maximum accepted limit of quantification (LOQ) based on food fat content (ranging from 0.1 to 10 mg/kg), recovery (70-120%), and intermediate-precision (10-20%) (Bauwens, Pantó & Purcaro 2021). As well as with other EVO matrices, Table 4 has a brief summary on analytical method chosen to determine MOSH/MOAH.

In LC-GC-FID, the distribution of molecular weight was associated with the breadth of a hump, which occurred at a certain reaction time owing to molecule elution (Srbinovska et al. 2020). Retention and selectivity in a packed column are strongly dependent on the mobile phase's solubility and the interaction and displacement of analytes from the stationary phase by the mobile phase (García-Cicourel et al. 2020). Continuity to GC and detector create chromatographic profile identification, counter-verified with certified reference material.

Thus, selection of internal verification standard is crucial. For MOSH, cyclohexyl cyclohexane (Cycy) is used as a suitable internal standard for mineral oil analysis due to its absence in significant quantities in mineral oils and packaging. Also, to control standard loss, n-C11 can be added in the same amount as Cycy, as it is more prone to volatilization. If the n-C11 area is smaller than Cycy, it may indicate Cycy loss or co-elution. n-C13, at half the amount of Cycy, is also added to verify chromatographic separation. Meanwhile, For MOAH analysis, 1- and 2-methylnaphthalene serve as internal standards due to their close elution, with equal peak areas confirming the absence of co-elution issues. n-Pentyl benzene is used to monitor volatile losses. While 1,3,5-tri-tert-butyl benzene (TBB) was traditionally a marker for the start of the MOAH fraction, di(2-ethylhexyl) benzene (DEHB) has proven to be more suitable, as it elutes with the first MOAH. Both DEHB and perylene can verify the boundaries of the MOAH fraction (Hoekstra, Bratinova & (Editors) 2019).

Gómez-Coca et al., (2016) established repeatability at the 50 mg/kg level by assessing oil sample containing 51 mg/kg with standard deviation (SD) of 1.9 and a variation coefficient of 4.0%. The LOD was calculated using "blank" refined sunflower oil supplemented with 10 mg/kg MO. While calculating the lowest analyte concentration, 0.5 mg/kg was used as LOD for both MOSH/MOAH as per the JRC limit guideline (Ruiz et al. 2021). Same was revealed by experimenting 152 sample originating from few countries and 12% of the samples tested positive for MOAH, with values ranging from 0.63 mg/kg to 82 mg/kg. Meanwhile, MOSH levels were identified above the LOQ in 92% of the goods, ranging from 0.5 mg/kg to 1152 mg/kg. As per investigation by Albendea et al. (2024), the LC-GC-FID system offers a sensitive method for detecting MO, with LOQ ranging from 0.05 mg/kg for the loin to around 0.2 mg/kg for the back fat and lipid sources.

Spiking technique was used in method validation to determine characteristics such as recovery, accuracy (intra and inter-day), and low relative SD with the highest R2. Furthermore, the validation may be cross-checked using a separate platform that has high relevance; in this instance,

the analysis is completed parallelly with the MS detector, allowing quantitative and qualitative assessment of MOSH/MOAH. Further experiment by Purcaro (n.d.) says on reproducibility of <16% as to compare with JRC Guidance, that is 20%, but were chosen as reference and trueness between +10% and -20%. The high uncertainty and low trueness reported for markers may indicate that these single peaks were incorrectly assigned due to additional interferences.

In short, online linked LC-GC combination provides great repeatability, sample throughput, resilience, and sensitivity while minimising sample waste. As a result, it is regarded as the golden option for an analytical procedure that allows for the effective separation and detection of specific molecules. But it has its limitations too, and this is due to not all compounds are amenable to LC-GC analysis. Certain analytes may not volatilize well for the GC step, or they may degrade at high temperatures, making it challenging to analyze thermally unstable or highly polar compounds.

Moreover, FID is a non-selective detector, meaning it can detect compounds based on carbon content, but it does not provide structural information about the compounds (counting aromatic rings or degree of alkylation), unlike detectors such as mass spectrometry (MS) (EFSA, 2019; Matheson, 2023). Molecules which has a similar molecular structure to POH, polyalphaolefin (POA), and lipids are frequently overlooked due to structure similarity (Buijtenhuijs & Van de Ven 2019). This is because uncertainty of interpretation and integration of LC-GC-FID was estimated to be around 20% owing to baseline drift, huge hump, and removal of riding peaks on the top of humps. Additionally, high boiling point substances may coelute with column bleed, creating inaccuracy in quantification (Bauwens, Pantó & Purcaro 2021). To minimize overestimation or underestimation of MOSH/MOAH identification and quantification, the data should be interpreted by a qualified and skilled individual.

Other Methods

The online approach LC-GC is still in demand and works very well quantitatively by FID and structural characterisation by UV detection or mass spectrometry (MS) (García-Cicourel et al. 2020). However, this method requires time, expertise and difficult data interpretation (Wagner & Oellig 2019). According to Ali & Neha (2024), integrating HPLC and LC/MS in conjunction is an effective method for comprehensively analyzing edible oils. Due to its superior ability to separate and quantify specific constituents, HPLC offers information on the nutritional value and qualitative characteristics of oil. While LC/MS ensures the safety of the oil and compliance with regulatory standards, it also extends the examination to trace-level contaminants.

Apart from this, characterisation and quantification of MOSH/MOAH, from complex to single hump, can be performed using two-dimensional gas chromatography (GC-GC)-FID due to its high separation capability. However, GC-GC-MS is prefreable for compound verification because it

allows both structural characterisation and determination of the degree of substitution of the aromatic compound (Bratinova et al. 2020; Pirow et al. 2019). In a few cases, GC-GC-FID/MS is used, where GC-GC (polar x apolar medium) allows separation according to volatility and polarity, while FID is used for quantification and MS for structural identification (Hochegger et al. 2021; Purcaro et al. 2013).

Lately in November 2022, Biedermann et al. (2022) has proven that GC×GC which uses two columns with distinct stationary phases and thermal modulation with FID, able to provide higher compound separation and resolution, which improves analysis of complex combinations, MO in main food products. This method was verified with conventional LC-GC-FID, and demonstrates greater sensitivity and the capacity to measure values as low as 0.02 mg/kg in spiked samples with internal standard, indicating the potential to detect lower MOAH concentrations. Also, LC-GC-FID/MS has been recommended as a marker for determination based on reliable detection of the compound and its origin (Hochegger et al. 2021). However, this strategy is currently being debated for maximal selectivity and accuracy. Purcaro et al. (2013) employed large volume (LVI) GC FID following the same Ag-SPE fractionation phase to get quantitative results as the LC-GC procedure.

Furthermore, Hochegger et al. (2021) introduced LC-GC x GC-ToF/MS/FID in 2020, where multi-measurement is done in a single analysis, and the greatest part is the distinguishing of MOAH in percentage without any advanced LC separation. Later in the year 2021, the researchers created specific 2D software that allows for the integration of data from the platform's numerous components, providing that MOSH and MOAH measurement is reliable and precise (Bauwens, Pantó & Purcaro 2021). This approach was also used in Fang et al. (2023) to identify and characterize the volatile organic

compounds (VOCs) in samples in order to distinguish consumable products from their original source. The expensive cost of ToF/MS restricts its laboratory applicability, despite the fact that it may effectively capture spectra for precise peak identification and quantification in GC×GC (Cardoso et al. 2023).

Planar solid phase extraction (pSPE) was developed as for paper and cardboard matrices screening method. It was connected with densitometry in UV and fluorescence detector (UV-FLD) for rapid analysis. Despite its high cost, this technique can extract 20 samples in parallel and perform a rapid clean-up and a chemical screening (Wagner & Oellig 2019). Pesticides were separated from tea (consumable matrix) using thin layer chromatography (TLC) with MS (Oellig & Schwack, 2011), and the method was improved by Oellig & Schwack (2014), where high throughput planar SPE (HTpSPE) was used in microliter-flow injection analysis coupled with TOFMS approach for pesticide determination in food.

Out of scope, the Nuclear Magnetic Resonance (NMR) analytical approach was chosen to detect the presence of MOSH/MOAH and even perform quick screening, potentially replacing the MO mist test according to IP346 (Buijtenhuijs & Van de Ven 2019). Few researchers used the NMR approach to measure MO in cosmetic products without significant sample preparation unless additional or defined determination of material, such as zero interferences, was required (Lachenmeier et al., 2017; Weber et al., 2019). This is due to the instrument's ease of use and the lack of a high level of skill or expense. However, EVO has not been used as a sample matrix by NMR. In terms of innovative methodologies, Bunaciu et al. (2022) employed IR spectroscopy (absorbance research) to test paraffinic compound adulteration in edible oil, namely olive and maise oil, using commercial software that is both precise and time efficient.

Table 4. Brief on analytical method on MOSH/MOAH determination

Matrix	Analyte	Pre-treatment/SPE	Clean-up Solvent	Analytical Method	LOD/LOQ mg/kg	Reference
Olive Oil	MO	SAISG	hexane	GC-FID	0.07 / 0.21	(Wrona, Pezo & Nerin 2013)
Edible Oil	MOSH/MOAH	Silver nitrated silica gel stationary phase/ epoxidation	-	GC-FID	-	(Ruiz et al. 2021)
Vegetable Oil	MOSH	Deactivated Silver silica gel	-	GC-FID	5 / 15	(Gómez-Coca et al. 2016)
Vegetable oil	MOSH	Offline SPE (1% Ag-activated silica gel)	n-hexane	LVI-GC-FID	-	(Liu et al. 2017)
Sunflower oil	MOAH	-	hexane	HPLC-GC-GC-FID/MS	-	(Biedermann & Grob 2009)
Olive /corn oil	MOSH	-	-	Infrared	-	(Bunaciu, Fleschin & Aboul-enein 2022)
Edible oil / fats	MOSH/MOAH	Automated workflow- saponification and epoxidation	n-hexane	LC-GC-FID	- / 1	(Nestola 2022)
Fats	MO	microwave-assisted saponification (MAS) and epoxidation	n-hexane	online LC-GC-FID	/0.2	(Albendea et al. 2024)
Food Products	MOSH/MOAH	Silver silica solid-phase extraction (SPE) cartridge	n-hexane	LC-GC-FID/MS	/ 1.2	(Purcaro et al. 2013)
Rice, Pasta, Cornflakes	MOSH /MOAH	-	-		0.2 /	(Buijtenhuijs & Van de Ven 2019)
Paper and Cardboard	MOSH /MOAH	Planar SPE	n-hexane	UV/FLD	-	(Wagner & Oellig 2019)

*****Blue** – non-oil matrix but closely related with analysis part

4. Conclusion

To put it simply, EVO is prone to MO contamination, which is further classified into MOSH and MOAH, either purposely, because of thermal stability, which improves oil shelf life, or accidentally due to migration from packing or processing via gas phase, depending on the volatility of the compounds. As it has a significant impact on human health, there is an advisory limit of 13mg MOSH/kg and 0.5mg MOAH/kg in the vegetable oil category in 2022; thus, several analytical methods have been carried out; however, the LC-GC-FID technique has been highly adapted in favour of high sensitivity, reproducibility, and the ability to quantify compounds in the absence of a pure sample. To acquire quantification of MOSH and MOAH in edible oil, a specific variant of this approach needs precise sample preparation and separation column type. There is still room for improvement in determining MOSH/MOAH in consumable products, especially in base food.

5. Acknowledgements

6. References

Ahmad, H., Noor, A.M., Sabri, M.P.A., Ngteni, R. & Syed Hilmi, S.M.H. 2019. Mineral oil saturated hydrocarbon in crude palm oil-current status in Sime Darby palm oil mills. *Journal of Advanced Agricultural Technologies* 6(4): 299–303.

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): 1–16.

Ali, F. & Neha, K. 2024. HPLC and LC - MS in Analysis of Edible Oils and Fats. In Ahamad, J. & Nollet, L. M. L. (ed.). *Bioactive Compounds of Edible Oils and Fats*, pages 234–242. First Edit. Taylor & Francis Group.:

Andriukaitis, V. 2017. Commission Recommendation (EU) 2017/84: The monitoring of mineral oil hydrocarbons in food and in materials and articles intended to come into contact with food. *Official Journal of the European Union*, page.

Apedo, A. & Snow, N.H. 2019. Online LC–GC: The Ultimate "GC Connection." https://www.chromatographyonline.com/view/online-lc-gc-ultimate-gc-connection-0 [15 October 2023].

Bauwens, G., Pantó, S. & Purcaro, G. 2021. Mineral oil saturated and aromatic hydrocarbons quantification: Mono- and two-dimensional approaches. *Journal of Chromatography A* 1643(462044): 1–9.

Biedermann, M., Eicher, A., Altherr, T. & McCombie, G. 2022. Quantification of mineral oil aromatic hydrocarbons by number of aromatic rings via comprehensive two-dimensional gas chromatography: First results in food. *Journal of Chromatography Open 2* 100072: 1–14.

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: 8711–8721.

Biedermann, M. & Grob, K. 2009. Comprehensive two-dimensional GC after HPLC preseparation for the characterization of aromatic hydrocarbons of mineral oil origin in contaminated sunflower oil. *Journal of Separation Science* 32: 3726–3737.

Biedermann, M. & Grob, K. 2015. Comprehensive two-dimensional gas chromatography for characterizing mineral oils in foods and distinguishing them from synthetic hydrocarbons. *Journal of Chromatography A* 1375: 146–153.

Biedermann, M., Uematsu, Y. & Grob, K. 2011. Mineral oil contents in paper and board recycled to paperboard for food packaging. *Packaging Technology and Science* 24: 61–73.

Biopharma. (n.d.). The Ames test or bacterial reverse mutation test. https://www.eurofins.com.au/biopharma-services/genetic-toxicology/the-ames-test [16 July 2023].

Boogaard, P., Hedelin, A., Riley, A., Rushton, E. & Vaissiere, M. 2012. Use of the modified Ames test as an indicator of the carcinogenicity of residual aromatic extracts. Brussels.

Bratinova, S., Hoekstra, E., Emons, H., Hutzler, C., Kappenstein, O., Biedermann, M. & McCombie, G. 2020. The reliability of MOSH/MOAH data: a comment on a recently published article. *Journal of Consumer Protection and Food Safety* 15(3): 285–287.

Buijtenhuijs, D. & Van de Ven, B.M. 2019. Mineral Oils in food ; a review of occurrence and sources. Bilthoven.

Bunaciu, A.A., Fleschin, S. & Aboul-enein, H.Y. 2022. Determination of some edible oils adulteration with paraffin oil using infrared spectroscopy. *Pharmacia* 69(3): 827–832.

Cardoso, C.A.L., Caramão, E.B., de Castro, T.L.A. & Simionatto, E. 2023. Variation in the chemical composition of essential oils from Mangifera indica L. leaves by comprehensive two-dimensional gas chromatography. *Ecletica Quimica* 48(4): 27–36.

Carrillo, J.-C., Shen, H., Momin, F., Kral, O., Schnieder, H. & Kuhn, S. 2022. GTL synthetic paraffin oil shows low liver and tissue retention compared to mineral oil. *Food and Chemical Toxicology* 159: 112701.

Carrillo, J.C., van der Wiel, A., Danneels, D., Kral, O. & Boogaard, P.J. 2019. The selective determination of potentially carcinogenic polycyclic aromatic compounds in lubricant base oils by the DMSO extraction method IP346 and its correlation to mouse skin painting carcinogenicity assays. *Regulatory Toxicology and Pharmacology* 106: 316–333.

EFSA. 2012. Scientific opinion on mineral oil hydrocarbons in food. *EFSA Journal*, page.

EFSA, Arcella, D., Baert, K. & Binaglia, M. 2019. Rapid risk assessment on the possible risk for public health due to the contamination of infant formula and follow-on formula by mineral oil aromatic hydrocarbons (MOAH). *EFSA Supporting Publications*, page.

EFSA Panel, Schrenk, D., Bignami, M., Bodin, L., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L., Leblanc, J.C., Nebbia, C.S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Schwerdtle, T., Vleminckx, C., Wallace, H., Alexander, J., Goldbeck, C., Grob, K., Gómez Ruiz, J.Á., Mosbach-Schulz, O., Binaglia, M. & Chipman, J.K. 2023. Update of the risk assessment of mineral oil hydrocarbons in food. *EFSA Journal* 21(9): 1–143.

European Commission. 2022. Standing committee on plants, animals, food and feed section novel food and toxicological safety of the food chain.

Fang, X., Chen, Y., Gao, J., Run, Z., Chen, H., Shi, R., Li, Y., Zhang, H. & Liu, Y. 2023. Application of GC–TOF/MS and GC×GC–TOF/MS to Discriminate Coffee Products in Three States (Bean, Powder, and Brew). *Foods* 12(3123): 1–12.

Fengler, R. & Gruber, L. 2022. Migration of mineral oil hydrocarbons from contaminated paperboard into the food simulants Tenax and Sorb-Star—A comparison. *Packaging Technology and Science*: 603–620.

Finch, A., Eilers, G. & Harzke, F. 2006. Masking from mineral oil smell and scenting from mineral oils. Bremen (DE).

Fiorini, D., Paciaroni, A., Gigli, F. & Ballini, R. 2010. A versatile splitless injection GC-FID method for the determination of mineral oil paraffins in vegetable oils and dried fruit. *Food Control* 21: 1155–1160.

Fiselier, K. & Grob, K. 2009. Determination of mineral oil paraffins in foods by on-line HPLC – GC – FID : lowered detection limit ; contamination of sunflower seeds and oils. *European Food Research and Technology* 229: 679–688.

Flinn, R.A., Kehl, W.L., Schmid, B.K. & Stewart, M.M. 1965. Hydrogenation of mineral oils in the presence of amenor amount of a hydrogenating catalyst and a major amount of specific type of activated alumina.

Foodwatch. 2021. International tests of various food products for their contamination by mineral oil hydrocarbons (MOSH/MOAH).

Gamble, K.R. & Smith, D.W. 2009. Discrimination of '" Odorless "' Mineral Oils Alone and as Diluents by Behaviorally Trained Mice. *Chemical Senses* 34: 559–563.

García-Cicourel, A.R., van de Velde, B., Roskam, G. & Janssen, H.-G. 2020. Supercritical fluid chromatography as a rapid single-step method for the determination of mineral oil saturated and aromatic hydrocarbons in purified mineral oils for food and cosmetics applications. *Journal of Chromatography A* 1614: 460713.

García-Cicourel, A.R., van de Velde, B., Verduin, J. & Janssen, H.G. 2019. Comprehensive off-line silver phase liquid chromatography x gas chromatography with flame ionization and vacuum ultraviolet detection for the detailed characterization of mineral oil aromatic hydrocarbons. *Journal of Chromatography A* 1607: 460391.

Gharbi, I., Moret, S., Chaari, O., Issaoui, M., Conte, L.S., Lucci, P. & Hammami, M. 2016. Evaluation of hydrocarbon contaminants in olives and virgin olive oils from Tunisia. *Food Control*, page. Elsevier Ltd.:

Gómez-Coca, R.B., Cert, R., Pérez-Camino, M.C. & Moreda, W. 2016. Determination of saturated aliphatic hydrocarbons in vegetable oils. *Grasas Y Aceites* 67(2): e127.

Hochegger, A., Moret, S., Geurts, L., Gude, T., Leitner, E., Mertens, B., O'Hagan, S., Poças, F., Simat, T.J. & Purcaro, G. 2021. Mineral oil risk assessment: knowledge gaps and roadmap. Outcome of a multi-stakeholders workshop. *Trends in Food Science and Technology* 113: 151–166.

Hochegger, A., Wagenhofer, R., Savic, S., Mayrhofer, E., Washuttl, M. & Leitner, E. 2022. Combination of multidimensional instrumental analysis and the Ames test for the toxicological evaluation of mineral oil aromatic hydrocarbons. *Journal of Agricultural and Food Chemistry* 70: 16401–16409.

Hoekstra, E., Bratinova, S. & (Editors). 2019. Guidance on sampling , analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials. Luxembourg.

IARC. 2012. Mineral oils, untreated or mildly treated: Evaluation of carcinogenic risks to humans, No. 100F. *IARC Monographs*: 179–196.

Institute for Publication. (n.d.). Mineral oil hydrocarbons MOSH / MOAH. Berlin.

Isola, A., Carrillo, J., Lemaire, P., Niemela, H. & Steneholm, A. 2023. Lack of human-relevant adversity of MOSH retained in tissues : Analysis of adversity and implications for regulatory assessment. *Regulatory Toxicology and Pharmacology* 137: 105284.

Jaén, J., Domeño, C., Alfaro, P. & Nerín, C. 2021. Atmospheric Solids Analysis Probe (ASAP) and Atmospheric Pressure Gas Chromatography (APGC) coupled to Quadrupole Time of Flight Mass Spectrometry (QTOF-MS) as alternative techniques to trace aromatic markers of mineral oils in food packaging. *Talanta* 227: 1–9.

Jaén, J., Domeño, C. & Nerín, C. 2022a. Development of an analytical method for the determination of mineral oil aromatic hydrocarbons (MOAH) from printing inks in food packaging. *Food Chemistry* 397: 1–9.

Jaén, J., Domeño, C. & Nerín, C. 2022b. Development of an analytical method for the determination of mineral oil aromatic hydrocarbons (MOAH) from printing inks in food packaging. *Food Chemistry* 397.

Jin, H. 2015. Dielectric strength and thermal conductivity of mineral oil based nanofluids. Delft Ubinersity of Technology, Netherlands,.

Jin, H., Andritsch, T., Tsekmes, I.A., Kochetov, R., Morshuis, P.H.F. & Smit, J.J. 2014. Properties of mineral oil based silica nanofluids. *IEEE Transactions on Dielectrics and Electrical Insulation* 21(3): 1100–1108.

Klaus, E.E., Tewksbury, E.J. & Fenske, M.R. 1962. Preparation , properties , and some applications of super-refined mineral oils. *ASLE Transactions* 5(1): 115–125.

Kojic, B.D., Dugic, P., Tatjana, B. & Dugic, G. 2019. Influence of the primary and secondary oxidation inhibors on the oxidation stability of the mineral base oils. *16th International Conference on Tribology Serbiatrib '19*, page 573–581.

Lachenmeier, D.W., Mildau, G., Krause, A., Marx, G., Walch, S.G., Hartwig, A. & Kuballa, T. 2017a. Evaluation of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) in pure mineral hydrocarbon-based cosmetics and cosmetic raw materials using 1H NMR spectroscopy [version 1; referees: 2 approved, 1 approved with reserva. *F1000Research* 6: 682.

Lachenmeier, D.W., Mildau, G., Krause, A., Marx, G., Walch, S.G., Hartwig, A. & Kuballa, T. 2017b. Evaluation of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) in pure mineral hydrocarbon-based cosmetics and cosmetic raw materials using 1H NMR spectroscopy. *F1000Research* 6.

Liao, R., Hao, J., Chen, G., Ma, Z. & Yang, L. 2011. A comparative study of physicochemical, dielectric and thermal properties of pressboard insulation impregnated with natural ester and mneral oil. *IEEE Transactions on Dielectrics and Electrical Insulation* 18(5): 1626–1637.

Liu, L., Huang, H., Wu, Y., Li, B. & Ouyang, J. 2017. Offline solid-phase extraction large-volume injection-Gas chromatography for the analysis of mineral oil-saturated Hydrocarbons in commercial vegetable oils. *Journal of Oleo Science*: 1–10.

Lommatzsch, M., Biedermann, M., Grob, K. & Simat, T.J. 2016. Analysis of saturated and aromatic hydrocarbons migrating from a polyolefin-based hot-melt adhesive into food. *Food Additives and Contaminants - Part A*: 473–488.

Lorenzini, R., Biedermann, M., Grob, K., Garbini, D., Barbanera, M. & Braschi, I. 2013. Migration kinetics of mineral oil hydrocarbons from recycled paperboard to dry food : monitoring of two real cases. *Food Additives & Contaminants: Part A* 30(4): 760–770.

Lorenzini, R., Fiselier, K., Biedermann, M., Barbanera, M., Braschi, I. & Grob, K. 2010. Saturated and aromatic mineral oil hydrocarbons from paperboard food packaging: estimation of long-term migration from contents in the paperboard and data on boxes from the market. *Food Additives and Contaminants* 27(12): 1765–1774.

Martin, D. 2008. Evaluation of the dielectric capability of ester based oils for power transformers based oils for power transformers. The University of Manchester,.

Matheson, A. 2023. A Modern Mineral Oil Hydrocarbon Analysis. https://doi.org/10.56530/lcgc.eu.do5379f3.

Moret, S., Scolaro, M., Barp, L., Purcaro, G. & Conte, L.S. 2016. Microwave assisted saponification (MAS) followed by on-line liquid chromatography (LC)-gas chromatography (GC) for high-throughput and high-sensitivity determination of mineral oil in different cereal-based foodstuffs. *Food Chemistry* 196: 50–57.

Müller, A., Jovalekic, M. & Tenbohlen, S. 2012. Solubility study of different gases in mineral and ester-based transformer oils. *2012 IEEE International Conference on Condition Monitoring and Diagnosis*, page 937–940. Bali.

Nestola, M. 2022. Automated workflow utilizing saponification and improved epoxidation for the sensitive determination of mineral oil saturated and aromatic hydrocarbons in edible oils

and fats. *Journal of Chromatography A* 1682: 463523.

Oellig, C. & Schwack, W. 2011. Planar solid phase extraction — A new clean-up concept in multi-residue analysis of pesticides by liquid chromatography – mass spectrometry. *Journal of Chromatography A* 1218: 6540–6547.

Oellig, C. & Schwack, W. 2014. Planar solid phase extraction clean-up and microliter-flow injection analysis – time-of-flight mass spectrometry for multi-residue screening of pesticides in food. *Journal of Chromatography A* 1351: 1–11.

Oppermann, U. & Leitner, E. 2017. Non-intentionally added substances in food and food contact materials - determination of mineral oil hydrocarbons by LC-GC Technique.

Otunga, G.N., Maiyoh, G.K., Macharia, B.N. & Tuei, V.C. 2019. Transformer mineral oil ingestion induces systemic sub-acute toxicity in Wistar rats. *Heliyon* 5: e02998.

Parkinson, L. 2022. EC sets limit on MOAH in foods. https://www.foodpackagingforum.org/news/ec-sets-limit-on-moah-in-foods [15 August 2023].

Patel, A., Guo, H. & Iglesias, P. 2018. Study of the lubricating ability of protic ionic liquid on an aluminium-steel contact. *Lubricants* 6(66): 1–13.

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. Taylor and Francis Ltd.:

Polyakova, A., van Leeuwen, S. & Peters, R. 2022. Review on chromatographic and specific detection methodologies for unravelling the complexity of MOAH in foods. *Analytica Chimica Acta* 1234: 340098.

Purcaro, G. (n.d.). Excutive Summary : Main Sources of Moh Contamination and Entry Points Into the Cocoa Supply Chain. Gembloux.

Purcaro, G., Tranchida, P.Q., Barp, L., Moret, S., Conte, L.S. & Mondello, L. 2013. Detailed elucidation of hydrocarbon contamination in food products by using solid-phase extraction and comprehensive gas chromatography with dual detection. *Analytica Chimica Acta* 773: 97–104.

Rausch, M.K., Erikson, H., Tollefsen, G.E. & Kelly, T.W. 1981. White mineral oil made by two stage hydrogenation. United States.

Rawlings, A. V & Lombard, K.J. 2012. A review on the extensive skin benefits of mineral oil. *International Journal of Cosmetic Science* 34: 511–518.

Rossum, V., Gijsbertus & Johannes. 2022. Removal of unwanted mineral oil hydrocarbons.

Rouabeh, J., M'barki, L., Hammami, A., Jallouli, I. & Driss, A. 2019. Studies of different types of insulating oils and their mixtures as an alternative to mineral oil for cooling power transformers. *Heliyon* 5: e01159.

Ruiz, J.L.H., Liébanas, J.A., Vidal, J.L.M., Frenich, A.G. & González, R.R. 2021. Offline solid-phase extraction and separation of mineral oil saturated hydrocarbons and mineral oil aromatic hydrocarbons in edible oils, and analysis via gc with a flame ionization detector. *Foods* 10: 2026.

Sabrina, M. & Giorgia, P. 2022. Mineral Oil in food: State of the art. https://foodsafety4.eu/knowledge/articles/mineral-oil-in-food-state-of-the-art/.

Sdrigotti, N., Collard, M. & Purcaro, G. 2021. Evolution of hyphenated techniques for mineral oil analysis in food. *Journal of Separation Science* 44(1): 464–482.

Spohner, M. 2017. Study of dielectric properties of mineral oils and natural oils and methyl esters of natural oils. *19th IEEE International Conference on Dielectric Liquids, ICDL 2017*, page 1–4. Manchester, United Kingdom.

Srbinovska, A., Conchione, C., Lucci, P. & Moret, S. 2020. On-Line HP(LC)-GC-FID determination of hydrocarbon contaminants in fresh and packaged fish and fish products. *Journal of AOAC International* 0(0): 1–7.

Suwarno & Darma, I.S. 2008. Dielectric properties of mixtures between mineral oil and natural ester from palm oil. *WSEAS Transactions and Power Systems* 3(2): 37–46.

Toudja, T., Moulai, H., Nacer, A., Beldjilali, A., Khelfane, I. & Debche, A. 2014. Moisture and electrical discharges effect on naphthenic mineral oil properties. *IET Science, Measurement and Technology* 8(6): 588–594.

Tuei, V.C. 2023. Availability of vegetable edible oils and potential health implications in Kenya. *Open Access Library Journal* 10: e9756.

United States Pharmacopeial Convention. 2014. Safety data sheet : Mineral Oil. United States.

Wagner, M. & Oellig, C. 2019. Screening for mineral oil saturated and aromatic hydrocarbons in paper and cardboard directly by planar solid phase extraction and by its coupling to gas chromatography. *Journal of Chromatography A* 1588: 48–57.

Walaszek, Z., Hanausek, M. & Slaga, T.J. 2007. The role of skin painting in predicting lung cancer. *International Journal of Toxicology* 26: 345–351.

Weber, S., Schmidt, T., Schumacher, P., Kuballa, T., Mildau, G., Walch, S.G., Hartwig, A. & Lachenmeier, D.W. 2019. Quantification of mineral oil aromatic hydrocarbons (MOAH) in anhydrous cosmetics using 1H NMR. *Journal of Chemistry*: 10.

Weber, S., Schrag, K., Mildau, G., Kuballa, T., Walch, S.G. & Lachenmeier, D.W. 2018. Analytical methods for the determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH)—A short review. *Analytical Chemistry Insights* 13: 1–16.

Wright, J. (n.d.). The fundamentals of mineral base oil refining. https://www.machinerylubrication.com/Read/28960/mineral-oil-refining [14 July 2023].

Wrona, M., Pezo, D. & Nerin, C. 2013. Rapid analytical procedure for determination of mineral oils in edible oil by GC-FID. *Food Chemistry* 141: 3993–3999.

Yuliastuti, E. 2010. Analysis of dielectric properties comparison between mineral oil and synthetic ester oil. Delft University of Technology,.

Zoccali, M., Barp, L., Beccaria, M., Sciarrone, D., Purcaro, G. & Mondello, L. 2016. Improvement of mineral oil saturated and aromatic hydrocarbons determination in edible oil by liquid-liquid-gas chromatography with dual detection. *Journal of Separation Science* 39: 623–631.