



Determining the source of contamination with MOSH and MOAH in the production of finely chopped cooked sausages

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ABSTRACT

Testing for the presence of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) hydrocarbon fractions was carried out on finished products at a food business entity. On this occasion, the presence of the MOSH fraction beyond the limits of the customer's internal standard (2 mg/kg) was determined in finely chopped cooked sausages at the end of their shelf life; levels were 48.2 ± 9.37 mg/kg in sausage packed in polyamide casing and 8.41 ± 1.64 mg/kg in sausage packed in collagen casing. The meat batter was tested systematically and in a targeted manner, successively in the production steps of the finished product, showing that aromatic hydrocarbon levels were: after passing through the crusher (0.92 ± 0.18 mg/kg), microcutter (1.63 ± 0.32 mg/kg), cutter (1.14 ± 0.22), and filler (1.19 ± 0.23 mg/kg) machines. The raw materials used in the product were also tested (1.00 ± 0.19 mg/kg), as were the casings (28408.82 ± 5525.51 mg/kg polyamide casing and 25947.15 ± 5046.72 mg/kg collagen casing) into which the final product was filled. The obtained test results determined the casings were the source of contamination.

1. Introduction

Over the past twenty years, the presence of mineral oil hydrocarbons (MOH) in foods has become a growing concern due to potential health risks. These oils are commonly used throughout the food supply chain—during harvesting, production, storage, and transportation—and are sometimes included as food additives (Grob, 2018). These compounds were suggested as important contaminants of the human body, with possible routes of contamination including air inhalation, food intake, and dermal absorption (Lachenmeier et al, 2017).

MOH are a wide range of products deriving from petroleum distillation fractions (Purcaro et. al., 2024), containing intricate mixtures of isomers and which are classified into two main categories: mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH). Edible oils typically contain higher concentrations of MOH compared to most other food products (Brühl, 2022). Mineral oils (such as paraffinum liquidum or white oil), which consist of MOSH and MOAH, are widely applied in various consumer products such as medicines and cosmetics (Weber et. al., 2018).

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Current evidence indicates that contamination can occur at various points throughout the entire production process. MOH are commonly utilized in food contact materials and food additives. They consist of complex mixtures, such as straight and branched open-chain alkanes (paraffins), primarily alkylated cycloalkanes (naphthenes), and can be generally categorized as MOSH and MOAH (Biedermann and Grob, 2009).

Up to now, there has been no comprehensive study on MOH risk assessment for the Serbian population. Globally, there is a number of toxicological and risk assessment studies on MOHs (JECFA, 2002). Animal research indicates that MOSH can cause deposits and inflammatory responses in the liver of specific rat strains. In 2012, the European Food Safety Authority (EFSA) identified MOAH as containing carcinogenic compounds that may accumulate in human tissues and potentially lead to the formation of microgranulomas (EFSA, 2012). In accordance with Codex Alimentarius Committee on Contaminants in Foods—Foresight on emerging issues in food and feed safety relevant to contaminants (Codex Alimentarius, 2024), MOAH with three or more aromatic rings are associated with genotoxicity and carcinogenicity, and a 1 mg/kg limit has been set for foods with a high fat/oil content ($> 4\%$ fat/oil to $\leq 50\%$ fat/oil). Detecting MOH in food presents significant challenges and demands a thorough, multifaceted analytical approach to address the complex issues involved (Sdrigotti *et al.*, 2021).

2. Materials and methods

Sample testing was performed in accordance with the EU standard (European Commission, 2017). This European Standard outlines a highly efficient method for determining saturated and aromatic hydrocarbons (from C10 to C50) in vegetable fats, oils, and food products based on vegetable oils (it is not intended for other matrices), validated through laboratory testing. The gold standard method for MOSH-MOAH analysis (Weber *et al.*, 2018) utilizes online-coupled high performance liquid chromatography-gas chromatography-flame ionization detection (HPLC-GC-FID) analysis. It can also be employed to examine MOSH and MOAH. The method has been evaluated in a laboratory study involving both naturally contaminated

and spiked samples of vegetable oils, mayonnaise, and margarine, with MOSH concentrations ranging from 4 mg/kg to 197 mg/kg, and MOAH from 2 mg/kg to 51 mg/kg. Based on the study results, the method is suitable for detecting MOSH and MOAH concentrations above 10 mg/kg. If natural sources interfere, the mineral origin of MOSH and MOAH can be confirmed by examining the pattern using GC-MS.

Compositional information on MOH is primarily from gas chromatography (GC). GC of MOSH results in a pattern of unresolved peaks of unidentified components (even at the highest separation efficiency available) with n-alkanes and some predominant iso- and cycloalkanes on top. GC of MOAH results in a pattern of unresolved peaks with hardly any distinct peak on top. (EFSA, 2012).

3. Results

After receiving unsatisfactory test results for the presence of MOSH and MOAH in ready-to-eat (RTE) finely chopped cooked sausages, our laboratory instigated an investigation at the customer's request.

It is noticeable from Figure 1 that the limit of 2 mg/kg, i.e., the customer's internal standard for MOSH, was exceeded several times in the examined sausages. The most commonly occurring fractions were C20-C35 hydrocarbons.

Tests of the meat batter from different production stages showed that all sausage samples examined contained Σ MOSH/MOAH at levels below the permitted limit (Figure 2). However, the results of testing the content of MOSH/MOAH showed that the sum of these hydrocarbons in the casings significantly exceeded the limit of 2 mg/kg. The most abundant fractions were C16-C35. Indeed, the industrial casings harboured extremely high levels of contamination (polyamide casing 28408.82 ± 5525.51 mg/kg and collagen casing 25947.15 ± 5046 mg/kg). After filling the product batter into casing, during the sausage shelf life, there was an increase in the concentration of MOSH in the finely chopped cooked sausages, to 48.2 ± 9.37 mg/kg (for sausages packed in polyamide casing) and to 8.41 ± 1.64 mg/kg (for those packed in collagen casing). The obvious source of the MOSH in the meat product was due to migration of the compounds from the contaminated casing.

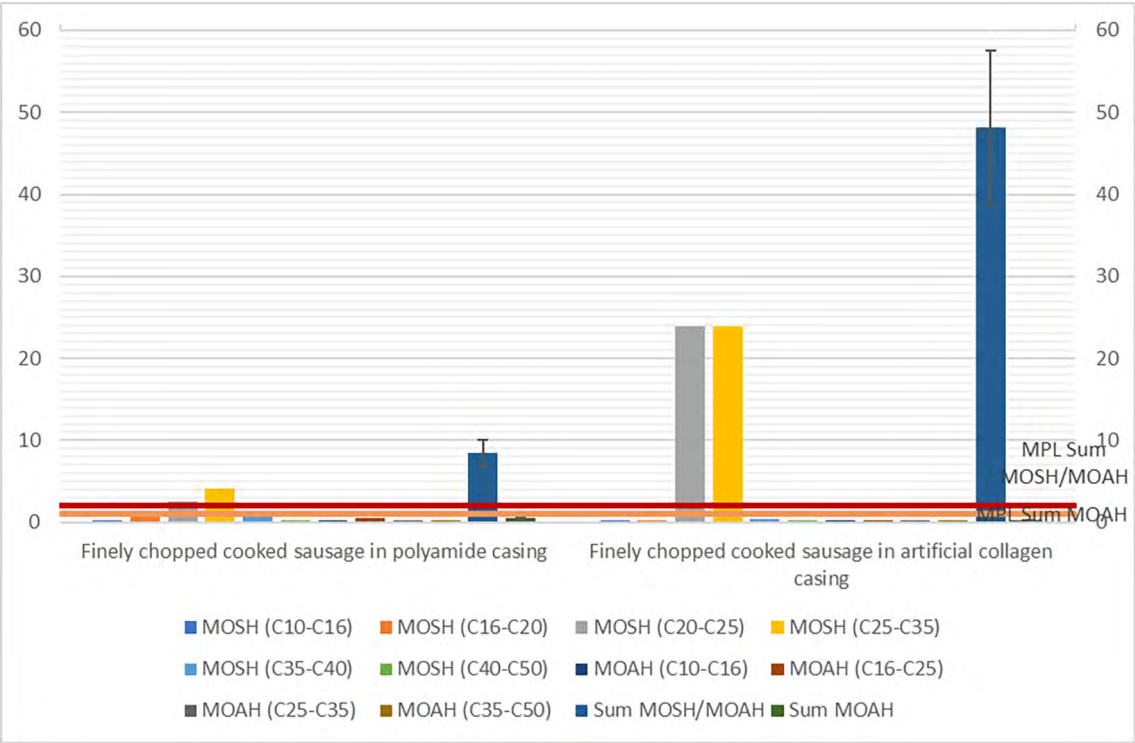


Figure 1. MOSH and MOAH measured in finely chopped cooked sausages packed in polyamide and in artificial collagen casings

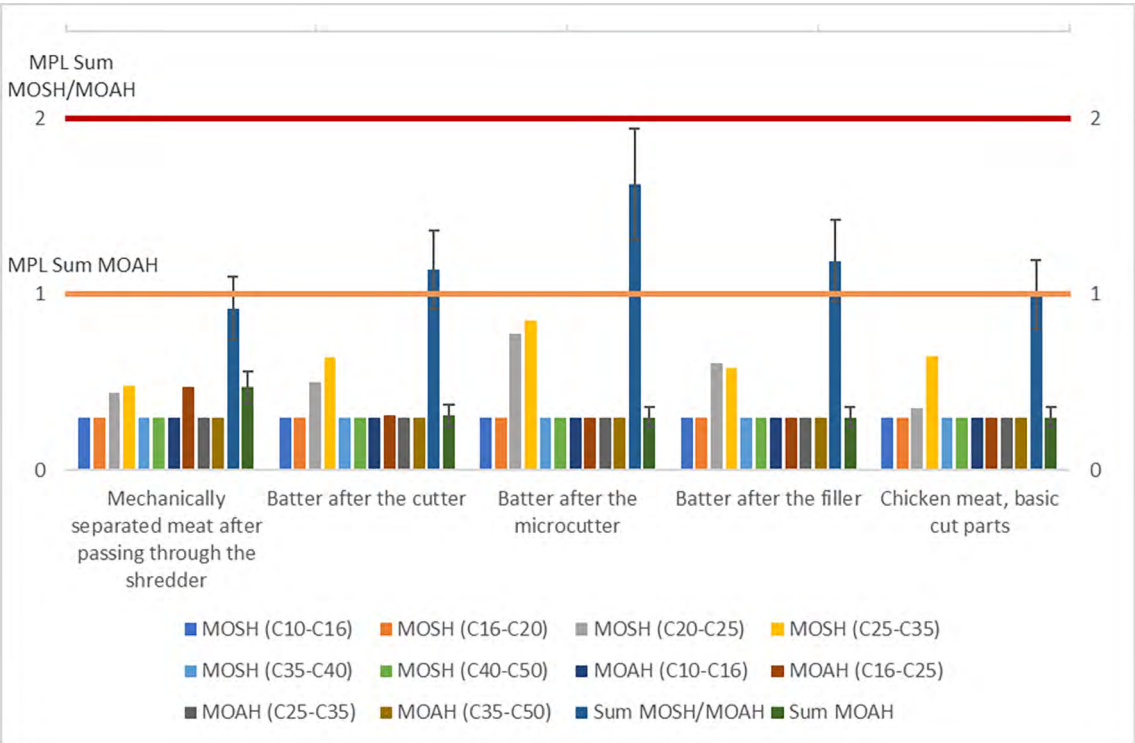


Figure 2. MOSH and MOAH measured at different production stages in the meat batter for the finely chopped cooked sausages

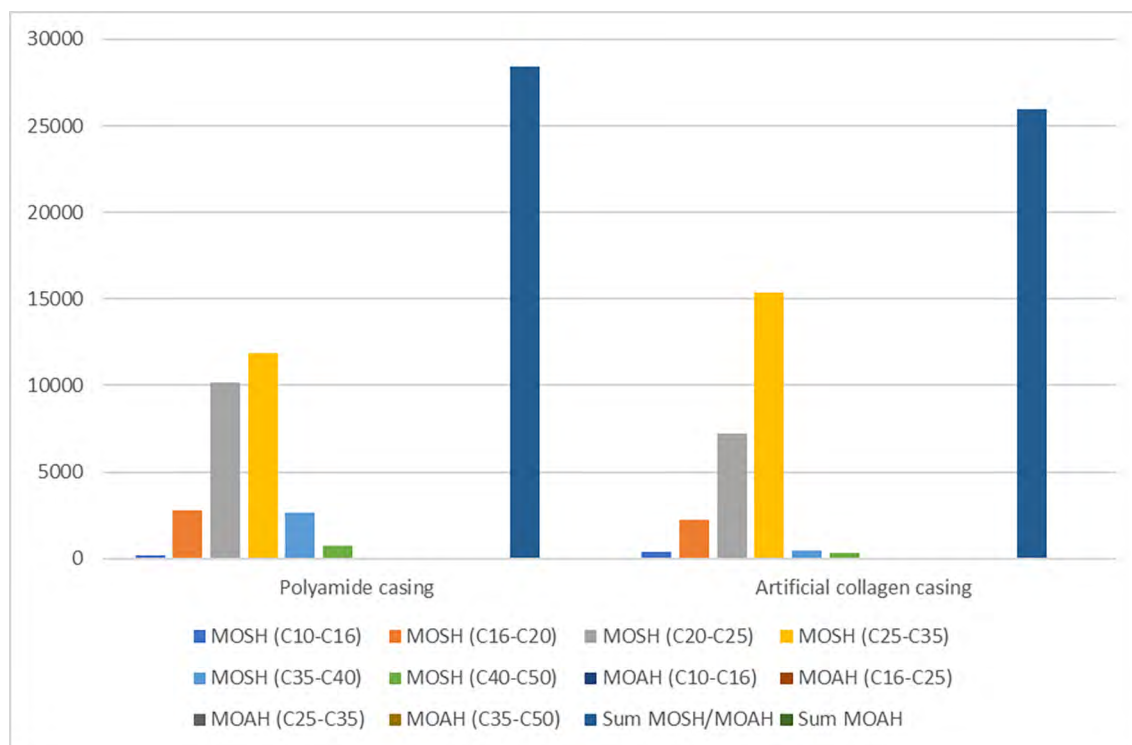


Figure 3. MOSH and MOAH measured in sausage casings (polyamide and artificial collagen)

4. Discussion

MOH are environmental and processing contaminants composed of a complex mixture of liposoluble compounds of petrogenic origin, containing between 10 and 50 carbon atoms (EFSA, 2023). Consumers are exposed to a range of MOH through food intake. According to EFSA's Scientific Opinion, MOAH may be carcinogenic and mutagenic (EFSA, 2012). Across the different age classes, the three food groups with the highest average contribution to the mean dietary exposure to MOSH were: grains and grain-based products; milk and dairy products and; animal and vegetable fats and oils and primary derivatives thereof (EFSA, 2023). Major sources of MOH in food are food packaging and additives, processing aids, and lubricants (EFSA, 2012). Another study found the highest contamination levels, measured in two beef rib samples, probably occurred during slaughter or handling prior to commercialization (Albendea and Purcaro, 2025).

There have been, and still are, discussions about the reliability of the results, particularly for measurements at low concentrations. The LC-GC-FID method can be considered as standard and reliable, validated by collaborative tests. Nonetheless, sometimes there large differences in results from different laboratories. There are several potential

reasons for this: blank and cross-contamination due to the ubiquitous presence of MOH; interference removal is a critical step at the lower limit of quantitation and; chromatogram interpretation needs experience. Reduction of uncertainty and analyst interpretation is fundamental to guarantee more reliable results (Purcaro, 2024)

Nuclear magnetic resonance (NMR) spectroscopy has recently been suggested for MOSH-MOAH analysis, especially with the possibility of detecting only the toxicologically relevant aromatic rings (Weber *et al.*, 2018). Moreover, carcinogenic, genotoxic, and mutagenic effects have been associated with compounds with three to seven aromatic rings (EFSA, 2012, 2023). For this subfraction, a surrogate reference point of 0.49 mg/kg body weight (b.w.) per day, calculated from data on eight polycyclic aromatic hydrocarbons, was considered (EFSA, 2023). Some of the compounds, such as benzene hydrocarbons, may be of potential health hazard, which specifically includes carcinogenic effects (Lachenmeier *et al.*, 2017).

In cosmetics, MOSH and MOAH occurred in 27 authentic samples, with contents in the range of 90-109 mg/kg and 0.02-1.10 mg/kg, respectively (Lachenmeier *et al.*, 2017).

In a survey in China, levels of MOAH and MOSH in food for infants and young children

were in the range of 0.50–1.34 mg/kg for MOAH, and 1.00–6.04 mg/kg for MOSH (Zhu *et al.*, 2019). MOSH from C16 to C35 may accumulate and cause microgranulomas in several tissues including lymph nodes, spleen and liver (EFSA, 2012). A mixture of highly branched alkanes and alkylated cycloalkanes was found to accumulate in different human tissues as liver or adipose tissue (Isola *et al.*, 2023).

The highest dietary exposure to MOSH was estimated for the young population, with lower bound–upper bound (LB–UB) means and 95th percentiles of 0.085–0.126 and 0.157–0.212 mg/kg bw per day, respectively. Considering a margin of exposure approach, EFSA concluded that the present dietary exposure to MOSH does not raise concern for human health for all age classes (EFSA, xxxx). Foodborne MOAH with three or more non- or simple-alkylated, aromatic rings may be mutagenic and carcinogenic, and therefore of potential concern. Revision of the existing acceptable daily intake for some food grade MOSH is warranted on the basis of new toxicological information (EFSA, 2012).

MOSH is generally considered of no concern at the concentration found, although accumulate in human body, while genotoxicity of MOAH with ≥ 3 aromatic rings is confirmed, and in the absence of reliable toxicity data, the dietary exposure to 1–2 ring MOAH might raise a concern (Purcaro *et al.*, 2024). The no-observed-adverse-effect level for

induction of liver microgranulomas by the most potent MOSH, 19 mg/kg b.w. per day, was used as a reference point for calculating margins of exposure (EFSA, 2012).

5. Conclusion

Generation of further data for the refinement of the risk assessment of MOSH and MOAH presence in food is needed. Current dietary exposure towards to MOSH for all age classes raises no concern for human health.

Our work revealed extremely high levels of MOSH contamination in the industrial casings used to package the sausages. Moreover, during product storage, the MOSH increased in the finely chopped cooked sausages to levels that greatly exceeded the producer's internal standard; we hypothesize this was due to migration from the contaminated casing.

Based on the levels of MOH in the tested finely chopped cooked sausages, this product was aligned with the Codex Alimentarius advice, according to which the concentration of the MOAH fraction in food with more than 4% fat should not exceed 1 mg/kg. However, the sausage product was not aligned with the manufacturer's specification, according to which the concentration of the MOSH fraction should not exceed 2 mg/kg.

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