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Determination of mineral oil aromatic hydrocarbons in edible oils and fats by online liquid chromatography–gas chromatography–flame ionization detection – Evaluation of automated removal strategies for biogenic olefins

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ABSTRACT

The determination of mineral oil aromatic hydrocarbons (MOAH) in foodstuffs gained in importance over the last years as carcinogenicity cannot be excluded for certain MOAH. The existence of olefins in foodstuffs, such as edible oils and fats, can be problematic for the determination of MOAH by LC-GC-FID. Removal of these interfering substances by HPLC based on polarity differences is not possible. During gas chromatographic separation heavily overloaded peaks are observed rendering the detection of small mineral oil contaminations almost impossible. Therefore, removal of these olefins is necessary before subjection of the sample to LC-GC-FID. Epoxidation of olefins to increase their polarity proved to be a valuable tool in the past. Precision and trueness of the results as shown in a collaborative trial, however, are relying on exact reaction conditions. Additionally, it is known that certain MOAH are oxidized during epoxidation and therefore get lost. In the scope of this work, hydroboration, bromohydrin reaction, and epoxidation were examined for their potential for derivatization of unsaturated hydrocarbons with increased robustness and higher recovery of MOAH. Epoxidation by *meta*-chloroperoxybenzoic acid (*m*CPBA) delivered the best removal of olefins. Factors influencing this reaction were enlightened. Adaption of the reaction conditions and time-controlled automation increased the recovery of polycyclic MOAH. Good precision (RSD_r <1.5%) and recovery (95–102%) for MOAH were also observed for sunflower and olive oils spiked with a lubricating mineral oil (at 24.5 mg/kg of MOAH). The trueness of the method was verified by analyzing collaborative trial samples.

1. Introduction

Hydrocarbons of mineral oil origin account for a large proportion of the known contamination in foodstuffs. According to an EFSA (European Food Safety Agency) opinion from 2012, MOH (mineral oil hydrocarbons) contribute also to a high degree to contamination found in the human body [1]. They can be categorized into two main groups: Saturated (MOSH) and aromatic hydrocarbons (MOAH) with variable alkyl chain lengths [2]. While the first group consists of paraffinic and naphthenic saturated hydrocarbons, the second one is composed of alkylated (possibly partially hydrogenated) aromatic hydrocarbons. The MOAH content of MOH can roughly range between 0 and 35% depending on the nature of the mineral oil [3]. While crude oils exhibit higher MOAH contents, refined hydrogenated oils show little to no MOAH contribution.

The presence of MOH contamination in foodstuffs can be attributed to several sources. Packaging material made from recycled paperboard (e.g. newspapers) that had been printed on with mineral oil derived ink is one important origin. Additionally, lubricants used during food processing, wax coatings directly applied to the food, environmental pollution, jute bags, etc. can be sources for contamination [4]. Found contaminations ranged from below 1 mg/kg up to several thousands of mg/kg [5]. In 2008, in Ukrainian sunflower oil more than 1000 mg/kg of mineral oil was found [6].

According to recent studies, acute toxicity upon oral intake of MOSH and MOAH is low [1]. Higher molecular MOSH are known to form microgranulomas in liver, spleen, lymph nodes, and other organs [1,7]. Because of the structural similarities to polycyclic aro-

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matic hydrocarbons (PAHs), some MOAH are suspected to have carcinogenic and mutagenic potential. It is known that alkylated PAHs, e.g., 1-methylpyrene, show increased carcinogenic potential compared to the parent compound (pyrene) [8]. *In vitro* assays gave indication that MOAH from printing ink have genotoxic potential [9].

Although no legislation is established till now for upper limits of MOSH and MOAH, minimization of both substance classes was advised by the EFSA and other national authorities such as the German Federal Institute for risk assessment (BfR) [10]. Upper limits of 0.6 and 0.15 mg/kg for MOSH and MOAH, respectively, were proposed in the past years derived from a temporary ADI (acceptable daily intake) of 0.01 mg/kg body weight (for a 60 kg person) and a suspected MOAH contribution of 25% [11]. In 2012, however, this ADI was withdrawn by the JECFA (Joint FAO/WHO Expert Committee on Food Additives) due to insufficient scientific data. In 2014, upper limits of 2.0 and 0.5 mg/kg for MOSH and MOAH, respectively, found in foodstuffs packaged in recycled cardboard were proposed in the latest draft for the 22nd amendment of the German consumer goods regulation [12]. In 2017, the upper limit for MOSH was removed from the draft and the use of a functional barrier enjoined [13].

1.1. Analytics of MOSH and MOAH

The determination of MOSH and MOAH is routinely performed by online HPLC-GC-FID. This method is based on a work by Biedermann et al. [14]. First publications regarding this topic and hyphenated HPLC-GC can be found already in the early 1990s [15].

Shortly, HPLC on bare silica gel is used for the separation of MOH components from the food matrix (lipids, sugars, etc.). Additionally, MOAH are separated from MOSH. The high capacity for retention of triglycerides allows the direct injection of edible oils after dilution [16]. Detection limits of approximately 5 mg/kg for MOSH and MOAH were reported for selected edible oils. For low-fat containing matrices, such as rice or pasta, detection limits as low as 0.5 mg/kg were feasible [14].

One remaining problem with certain matrices is the co-elution of biogenic olefins during the HPLC separation. Some monoterpenes are eluted in the MOSH fraction, while polyunsaturates, such as carotenes, squalene, and sterenes, can be found in the MOAH fraction. Because of their natural abundance, these compounds may form large peaks overloading the subsequent GC separation column hindering the detection of MOAH (see Fig. 1). Because of the low content found in edible oils and fats, the co-elution of monoterpenes etc. in the MOSH fraction is mostly negligible and therefore out of the scope of this work.

Zoccali et al. described a method capable of removing the polyunsaturates from MOAH by HPLC [17]. After a first cleanup on silica, the MOAH fraction was separated from the polyunsaturates on Ag^+-treated silica gel. To that end, a commercial silica HPLC column was flushed with silver nitrate [17,18]. Squalene from olive oil could be retained while MOAH with up to three aromatic rings were eluted in a transfer volume exceeding 2 mL. MOAH with larger ring systems were retained too strongly on the prepared column. The authors gave no information regarding the elution of sterenes or carotenes present in vegetable oils. Moreover, stability of silver-ion impregnated HPLC columns is known to be limited [19].

Independently, additional sample cleanup steps were developed [14,20,21]. Treatment of the sample with elemental bromine was used to derivatize the biogenic unsaturated hydrocarbons. Because of the toxicity of bromine and insufficient selectivity, epoxidation by *meta*-chloroperoxybenzoic acid (*m*CPBA) was proposed. Increased polarity of the polyunsaturates was the aim in both cases. Consequently, a removal of these compounds during the HPLC separation became feasible.

Typical reaction conditions for epoxide synthesis with *m*CPBA include the use of dichloromethane as solvent and possible sub-ambient cooling for improved selectivity [22]. Quenching of the reaction is normally done by washing the sample with a reducing agent such as sodium thiosulfate. Initial addition of sodium bicarbonate or a subsequent washing step was reported to improve the recovery of acid-labile epoxides. Ring opening could otherwise occur by catalytic amounts of *meta*-chlorobenzoic acid formed during the reaction [23].

The proposed reaction route by Biedermann et al. for the determination of MOAH in edible oils and fats consisted of addition of *m*CPBA in dichloromethane at sub-ambient temperatures, i.e., ice bath cooling [14]. The authors noticed that *m*CPBA also attacked certain MOAH constituents, such as polycyclic aromatic compounds (PAHs, thiophenes), due to its oxidation potential. Roughly 20% of MOAH in non-refined mineral oils were reported to be lost even at sub-ambient temperatures. Higher *m*CPBA amounts further increased the loss of MOAH. The presence of a food matrix containing large amounts of unsaturated fatty acids was found to be beneficial for the recovery of MOAH. Unsaturated fatty acids were reported to be attacked prior to MOAH compounds. Therefore, addition of uncontaminated edible oil as buffering agent was recommended for samples containing only small amounts of unsaturated fatty acids [14]. This method is most widespread and established in routine environments.

However, a collaborative trial showed high variances in the obtained results (z-scores >2) possibly originating from varying reaction conditions [24]. During routine application, it is often seen that the recovery of the ISTD used for quantitation of MOAH is diminished by epoxidation resulting in overestimation of the results. For use in routine environments, method robustness is yet insufficient and needs to be improved.

For these reasons, aim of the current work was to explore possibilities for removal of polyunsaturates from the MOAH fraction offering increased robustness. Therefore, derivatization of the polyunsaturates was further explored. Hydroboration and bromohydrin reaction were examined for their suitability for removal of polyunsaturates. Investigation of the reaction conditions of *m*CPBA epoxidation enabled an optimization and automation of this technique representing an important achievement to increase method robustness.

2. Materials and methods

2.1. Samples

Extra virgin olive oil and refined sunflower oil were obtained at the local supermarket and used for method development and validation. Additionally, edible oil samples from a collaborative trial performed in 2015 within the CEN/TC275/WG13 work program (European Committee for Standardization) organized by ITERG (Pessac, France) were available. They consisted of refined olive pomace oil, extra virgin olive oil, and palm oil.

2.2. Chemicals and solutions

Acetone, acetonitrile, dichloromethane, ethanol, and *n*-hexane were from LGC Promochem (Picograde quality, Wesel, Germany). The internal standard (ISTD) for MOH quantitation (Cat. No. 31070 – *n*-undecane, *n*-tridecane, bicyclohexyl, α-cholestane, *n*-pentylbenzene, 1-methylnaphthalene, 2-methylnaphthalene, 1,3,5-tri-*tert*-butylbenzene, perylene) and an EPA-PAH standard (Cat. No. 31011) were supplied from Restek (Bellefonte, PA, USA). A lubricating oil standard (K009) for spiking experiments was obtained from the Federal Institute for Materials Research