WEJ Contaminants

Determination of mineral oil aromatic hydrocarbons (MOAH) in food by LC GC FID – Comparison of conventional vs. automated epoxidation

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Introduction

Hydrocarbons of mineral oil origin (MOH) are an important and widespread contamination in foodstuff [1]. These contaminants can be categorized in two main groups: mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH). MOSH consist of linear and branched alkanes, and alkyl-substituted cycloalkanes. While MOAH comprise of alkylated aromatic hydrocarbons. Several sources could be responsible for the MOH contamination of foodstuff, like lubricants used during food processing, recycled paperboard, jute bags or environmental pollution [2]. So, the determination of MOH in foodstuff gained importance over the last years. Especially, due to the potential carcinogenicity of certain MOAH.

MOAH analysis of foodstuffs by LC-GC-FID without clean-up is complicated by the presence of various interfering substances, such as squalene, sterenes and carotenoids. These substance classes co-elute with the MOAH fraction and overload the GC-column which renders the detection of small mineral oil contaminations almost impossible. Removal of these interferences by HPLC based on polarity differences is not possible. Epoxidation of interferences to increase their polarity seems to be a good possibility. Therefore, epoxidation by meta-chloroperoxybenzoic acid (m-CPBA) provided the best removal of interfering substances. By the conventional epoxidation, dichloromethane as solvent and sub-ambient cooling for improved selectivity is used; the reaction is stopped by sodium bicarbonate [3,4]. This method is widespread and established in routine analysis for MOAH. But conventional epoxidation is complex and did not give suitable results for all matrices respectively interferences.

Epoxidation using n-hexane was inefficient as solubility of m-CPBA was limited. Due to this fact, Nestola et al. [5] added ethanol to the reaction as it offered several advantages: solubility and stability of m-CPBA in ethanol proved to be well suited for an automated approach; ethanol and n-hexane were miscible in any proportion; removal of ethanol from the n-hexanic sample was possible by addition of water; and the reaction kinetics could be adjusted by tweaking the amount of ethanol in the reaction mixture. Therefore, sub-ambient cooling was no longer necessary as ethanol delayed the reaction sufficiently and provided a higher selectivity and robustness even at room temperature. This method showed good precision and recovery for MOAH. Furthermore, the adaption of the reaction conditions and time-controlled automation increased the recovery of MOAH [5]. Matrix interferences were enormously reduced and the results were comparable to the routine method. This represents an important step forward for routine analysis.

Method

The sample was weighed into a extraction vessel and internal standard was added. The sample was extracted with n-hexane. For enrichment and purification, an aliquot of the extracted sample were given on a glass column filled with activated silica gel and eluted with n-hexane/dichloromethane. The resulting eluat was concentrated and filled in a 10-ml autosampler vial. The vial was placed onto the autosampler, which performed all subsequent steps fully automated.

As first step, the autosampler added 500 µl of an ethanolic m-CBPA solution (200 mg/ml) to the sample. The vial was placed into an agitator and was shaken at a speed of 500 rpm for 15 min at room temperature. Afterward, 500 µl of ethanol and 2 ml of an aqueous sodium thiosulfate solution (100 mg/ml) were added to destroy excess m-CPBA and induce phase separation. The additional amount of ethanol was added to enable phase separation. The vial was shaken at 750 rpm for 30 s and then centrifuged. Five hundred microliters of the n-hexanic upper phase were transferred into a 2-ml autosampler vial prefilled with a spatula tip of sodium sulfate. The dried organic phase was subjected to LC-GC-FID.

LC-GC-FID experiments were performed on a system from Axel Semrau (Sprockhövel, Germany). It consisted of a 1260 Infinity HPLC system (binary pump and variable wavelength detector), 7890B GC with flame ionization detector, both from Agilent Technologies (Waldbronn, Germany), and a DualPAL autosampler (CTC Analytics AG, Zwingen, Switzerland). The CHRONECT LC-GC interface module is the connecting element by Axel Semrau.

Figure 1 LC-GC-FID system by Axel Semrau equipped with automated epoxidation.

Results

The obtained repeatability was better than 1.1 % and the recovery ranged from 94 to 103 % including the internal standards.

Spiked maize oil was prepared with the conventional and automated epoxidation. Figure 2 shows the conventional epoxidation and figure 3 the new method. In the case of maize oil, almost no interfering peaks could be detected after epoxidation with the automated method, only a few sharp peaks could be observed.

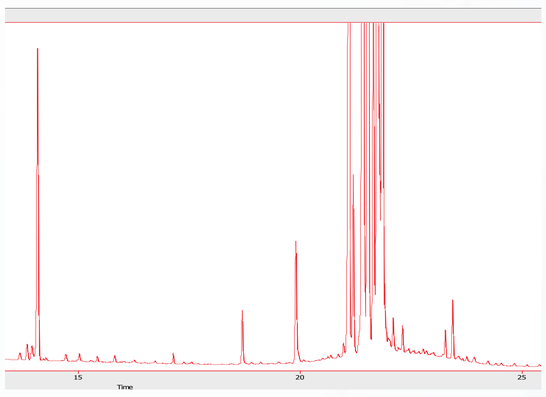

Figure 2 LC-GC-FID chromatogram of the MOAH fraction of maize oil prepared with conventional epoxidation.

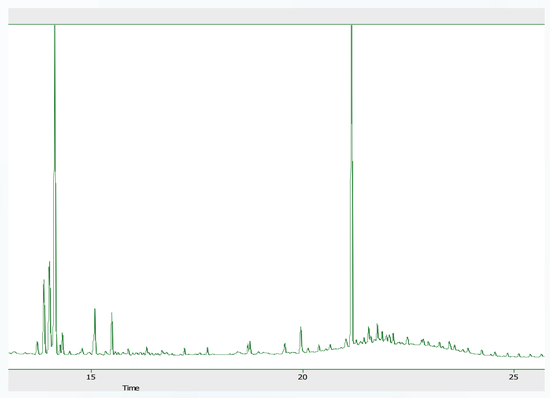

Figure 3 LC-GC-FID chromatogram of the MOAH fraction of maize oil prepared with new epoxidation.

For olive oil, no traces of residual squalene were found after the new method (see fig. 4). With the conventional epoxidation, squalene can be still detected (see fig. 5).

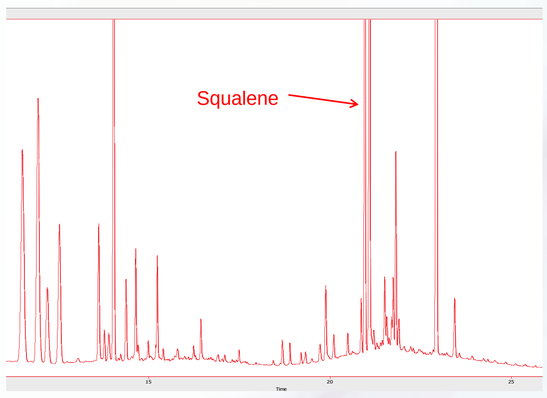

Figure 4 LC-GC-FID chromatogram of the MOAH fraction of olive oil prepared with conventional epoxidation.

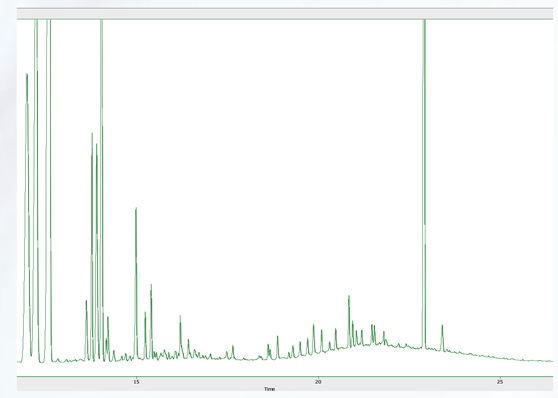

Figure 5 LC-GC-FID chromatogram of the MOAH fraction of olive oil prepared with automated epoxidation.

Despite of the better epoxidation of interfering substances with the new method, the results for automated and conventional epoxidation were comparable (see table 1). Furthermore, the new, automated epoxidation can prevent incorrect interpretations caused by interferences in the chromatogram.

Table 1 Comparison of automated and conventional epoxidation.

	MOAH total [ppm]	
	conventional	automated
maize oil	11,7	11,7
olive oil	7,4	7,8

Conclusion

The epoxidation delivered good and comparable results for almost all matrices and could be a suitable alternative to the conventional epoxidation. Substitution of the reaction solvent made the use of sub-ambient temperatures obsolete. Solvent evaporation and sample reconstitution were no longer necessary either. MOAH could be quantitatively recovered in absence of a matrix, which is related to retarded reaction kinetics and the use of an efficient quenching step [5]. This represents an important achievement in comparison to the established method.

This method showed good precision and recovery for MOAH. Furthermore, the adaption of the reaction conditions and time-controlled automation increased the recovery of MOAH. Matrix interferences were enormously reduced by this epoxidation method and the results were comparable to the routine method and the trueness of the method was verified by analyzing collaborative trial samples.

Literature

[1] L. Barp, C. Kornauth, T. Wuerger, M. Rudas, M. Biedermann, A. Reiner, N. Concin, K. Grob, Food Chem. Toxicol. 2014, 72, 312–321. [2] M. Biedermann, K. Grob, J. Chromatogr. A 2015, 1375, 136–153. [3] M. Biedermann, K. Fiselier, K. Grob, J. Agric. Food Chem., 2009, 57, 8711–8721. [4] CEN/TC 275 N 1069. [5] M. Nestola, T. C. Schmidt, J. Chromatogr.. A, 2017, 1505, 69-76

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