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Food Safety Analysis

Refined Risk

In the refining process for edible oils conditions must be carefully controlled to avoid the formation of toxic process contaminants, such as 2-MCPD, 3-MCPD, glycidol and their fatty acid esters. An automated GC/MS-method now enables highly efficient determination of these compounds based on standard methods such as ISO 18363-1, AOCS Cd 29c-13, and DGF C-VI 18 (10).

By Guido Deussing

The European Food Safety Authority (EFSA) has recently updated their risk assessment for 3-monochloropropanediol (3-MCPD), 2-MCPD, fatty acid esters of these compounds, as well as glycidyl fatty acid esters (GE) in food [1]. The result is based on data submitted by 23 member states: "Glycerol-based process contaminants found in palm oil, but also in other vegetable oils, margarines and some processed foods, raise potential health concerns for average consumers of these foods in all young age groups, and for high consumers in all age groups." [2]. According to the press release: "The highest levels of glycidyl esters, as well as 3-MCPD and 2-MCPD (including esters) were found in palm oils and palm fats, followed by other oils and fats. For consumers aged three

and above, margarines and 'pastries and cakes' were the main sources of exposure to all substances." Incidentally, the FDA refers to GE as glycidol fatty acid esters.

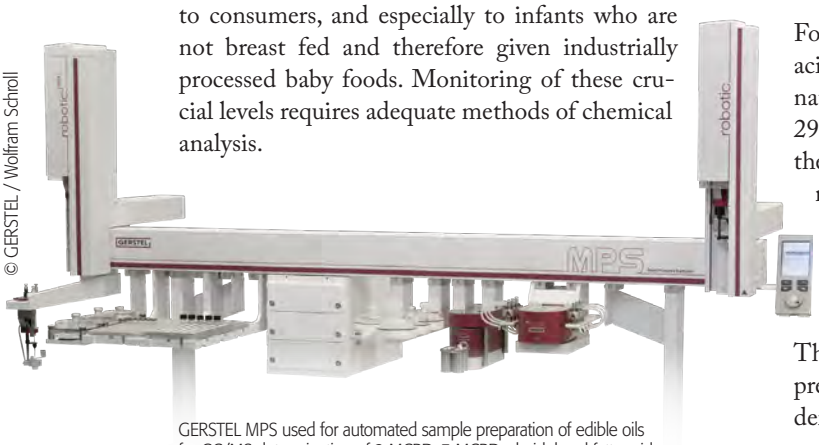
Glycerol as building block

All fats and edible oils contain glycerol in the form of fatty acid esters (triglycerides). Not all oils are ready for consumption in their native form; processing is generally required to remove off-odors and to ensure sufficient shelf life. For deodorization, as part of the refining process, steam is used to gradually heat the oil under vacuum to around 200-230 °C to ensure that unwanted flavor and taste-intensive compounds are removed along with other unwanted VOCs and even pesticides. When chloride is present, heat treatment accelerates the substitution of fatty acids in triglycerides with chlorine atoms, leading to the formation of 2-MCPD and 3-MCPD fatty acid esters. Under these conditions, diglycerides also react to form the glycidyl fatty acid esters.

Assessing the risk

The EFSA risk assessment of the above-mentioned glycerol derivatives is based on findings from animal experiments: In rats that had been fed 3-MCPD, cell changes were found, especially in the kidney area. Higher dosages led to benign tumors, as reported by the BfR in Berlin. According to the EFSA, the tolerable daily intake (TDI) value is 0.8 µg per kg body weight for 3-MCPD; due to a lack of adequate toxicological information, no assured TDI value for 2-MCPD can be given.

The risk assessment of glycidyl fatty acid esters is based on the assumption that these are fully transformed to free glycidol in the body. Since the free compound is known to be both genotoxic and carcinogenic, the experts from the panel on contaminants in the food chain (CONTAM)[3] organized by EFSA could not provide any guidance on a safe level of glycidyl fatty acid esters. When a safe level doesn't appear to exist, there is all the more need for action toward minimizing the level of these contaminants in order to minimize any health risk to consumers, and especially to infants who are not breast fed and therefore given industrially processed baby foods. Monitoring of these crucial levels requires adequate methods of chemical analysis.



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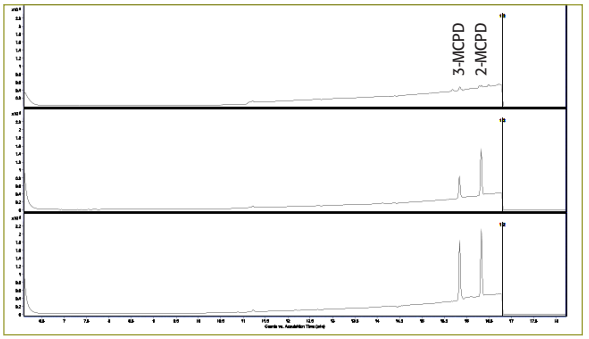
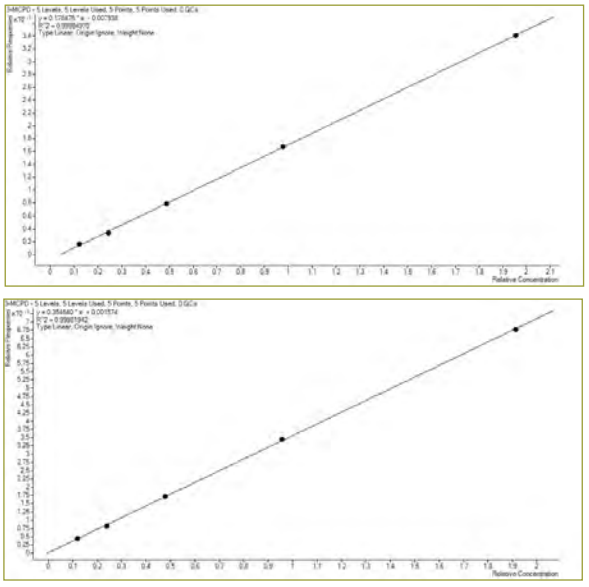
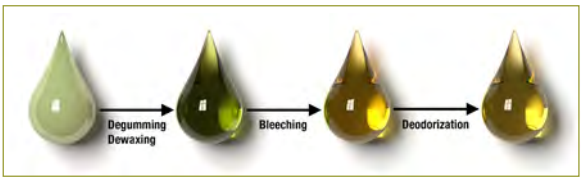
GERSTEL MPS used for automated sample preparation of edible oils for GC/MS determination of 2-MCPD, 3-MCPD, glycidol and fatty acid esters of these.



SIM chromatogram m/z 198 : Top: Virgin olive oil used as blank oil. Middle: Edible oil sample assay B (3-MCPD). Bottom: Edible oil sample assay A (3-MCPD + glycidol).



Linearity study for 3-MCPD assay B (top) and glycidyl assay A (bottom), 0.12-1.9 mg/kg each.



Refining process for production of edible oils.

A challenge for the analytical chemist

For the determination of 2-MCPD and 3-MCPD fatty acid esters, as well as glycidyl fatty acid esters, internationally, the ISO 18363-1 [4] and the AOCS Cd 29c-13 [5] methods are widely accepted. In Germany, the German Society for Fat Sciences (DGF) recommends a unified method, DGF C-VI 18 (10) [6]. The mentioned methods are all based on the use of gas chromatography with mass spectrometric detection (GC/MS). The DGF unified method C-VI 18 (10) is almost identical to the AOCS Cd 29c-13 method: The 3-MCPD content of the 3-MCPD fatty acid esters present is determined following alkaline hydrolysis and derivatization with phenylboronic acid (assay B). These methods also enable determination of glycidyl esters (as

bound glycidol) by indirect calculation after hydrolysis and conversion to 3-MCPD. Glycidol is determined as the difference between the total amount of 3-MCPD present including converted glycidol (assay A) and the amount of 3-MCPD determined in assay B. This in short highlights the added value of the unified DGF C-VI 18 (10) and the AOCS Cd 29c-13 methods. These methods enable the determination of the amount of glycidol in the sample and reading through them provides a fair impression of the effort required to transform the compounds such that they can be determined by gas chromatography, in this case relying on a manual sample preparation process that includes hydrolysis, extraction and derivatization. In addition, duplicate analysis of each sample prepared in two different ways is needed in order to determine both 3-MCPD and glycidol.

Higher efficiency and better sensitivity through automation

GERSTEL experts have developed a fully automated sample preparation solution, integrated with the GC/MS system, that replicates the AOCS Cd 29c-13/ DGF C-VI 18 (10) methods step by step on a robotic sampler. "The automated sample preparation is performed on a Multi-Purpose Sampler (MPS robotic*pro*) Dual Head version with two independently moving towers fitted with different syringe sizes. This means that larger amounts of liquid can be handled for sample preparation with one syringe while another smaller syringe handles smaller volumes, for example, used for injection into the GC/MS", says Dominik Lucas, formerly Application Specialist, now member of the German sales organization within GERSTEL.

In addition, method steps such as liquid-liquid extraction, solvent evaporation, solvent exchange to a GC-compatible solvent and analyte derivatization, are fully automated and very efficiently integrated in the overall method (see listing on the right hand side). Finally, the MPS introduces the prepared sample to the GC/MS system. Dominik Lucas adds: "The analysis results we generated using the described automated method showed good correlation with reference analysis results obtained from independent laboratories. Relative standard deviations for repeat analyses were at 5 percent for 3-MCPD and 6.4 percent for glycidol for the complete process". As to using the described sample preparation and system solution for determination of 2-MCPD, 3-MCPD, glycidol and their fatty acid esters: "The evaporation step in the method means that users can reach the required sensitivity and stability even when using a single quadropole mass spectrometer", Dominik Lucas states. The evaporation step ensures better sensitivity by concentrating analytes. In addition, it provides improved overall system stability by removing excess derivatization reagent before it can enter and destabilize the MS ion source.

MANUAL

- Weigh a 100 mg sample into a vial
- Fill a second vial with sodium sulfate as drying agent (drying vial) – optional

GERSTEL MPS

- Add MTBE to the sample
- Add ISTD solution and mix, or melt and mix (solids)
- Add MeOH/NaOH mixture
- Agitate and incubate
- Add acidic NaCl solution (Assay A)
- Add acidic NaBr solution (Assay B)
- Add n-hexane for matrix extraction
- Agitate and incubate
- Discard hexane phase
- Repeat extraction with n-hexane twice
- Perform multiple analyte extractions using MTBE/Ethylacetate 3:2 (v/v), transfer the organic phases to the drying vial
- Add phenylboronic acid solution
- Evaporate to dryness and derivatize in the ^{m}VAP at 50 °C and subambient pressure
- Take up the derivatives in isooctane
- Introduction to GC/MS(/MS) if integrated with sampler

Listing of the required manual sample preparation steps, which were transferred to the GERSTEL MPS and automated. The MPS automates the AOCS Cd 29c-13 and DGF C-VI 18 (10) methods for the determination of 2-MCPD, 3-MCPD, glycidol and fatty acid esters of these. Depending on the instrument configuration, the prepared extracts can be introduced directly to the GC/MS system for analysis.

References

[1] www.efsa.europa.eu/en/efsajournal/pub/4426
[2] www.efsa.europa.eu/en/press/news/160503a
[3] www.efsa.europa.eu/en/panels/contam
[4] ISO 18363-1:2015 Animal and vegetable fats and oils -- Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS -- Part 1: Method using fast alkaline transesterification and measurement for 3-MCPD and differential measurement for glycidol
[5] AOCS Official Method Cd 29b-13, Revised 2017: 2- and 3-MCPD Fatty Acid Esters and Glycidol Fatty Acid Esters in Edible Oils and Fats by GC/MS (Difference Method)
[6] DGF Unified Method C-VI 18 (10) (Available only in German language).