Challenges in Analysing Glycidyl Fatty Acid Esters in Edible Oils and Fats

Comparison of results achieved with indirect methods and single ester analysis

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Introduction

Glycidyl fatty acid esters (GEs) have initially been identified in refined palm fats as process contaminants followed by their detection in many vegetable fats, oils and fat-containing products including infant formulas. Glycidol has mutagenic and carcinogenic properties and was classified by IARC as "probably carcinogenic to humans" (2A); therefore, the availability of reliable analytical methods is mandatory for consumer health protection purposes.

Two general approaches for the analysis of GE in fats and oils are applicable: Direct determination of the various GEs and indirect methods which comprise hydrolyzation of the esters and further transformation of the intermediate glycidol into a substance like 3-MCPD which can be distinctly measured. For both direct and indirect methods we investigated parameters affecting the quantification of GEs in different samples to a greater or lesser extent.

Materials and Methods

In this project indirect determination of GEs was carried out using GC-MS according to a standard method developed by Deutsche Gesellschaft für Fettwissenschaft 2011 [1], Kuhlmann 2011 [2] and Fiebig 2011 [3]. All investigated indirect methods are based on alkaline catalyzed ester cleavage and derivatization with phenyl boronic acid. All of them take account of the presence of 3-MCPD esters in the sample by analyzing the samples twice with two different treatments.

Results for direct GE determination were achieved on the basis of a double solid-phase extraction method followed by LC-MS detection developed by Shiro et al. [4].

Indirect Methods - Alkaline Catalyzed Release of Glycidol from its Individual Fatty Acid Esters

Method 1: In assay A, the sample is treated with sodium chloride in acidic solution – glycidol released from GE reacts with inorganic chloride to 3-MCPD. In assay B, a second aliquot of the sample is treated with an acidic chloride-free salt solution (e.g. sodium bromide) for the determination of 3-MCPD originating from 3-MCPD esters. For the calculation of glycidol the difference of both MCPD contents (Assay A and B) is multiplied by a transformation factor, representing the amount of 3-MCPD generated in the presence of chloride from glycidol which must be considered as due to conditions occurring during sample preparation [1].

Applying Method 2, samples are treated with chloride-free salt solution (e.g. sodium bromide). The quantification of glycidol (originating from GEs) and 3-MCPD (from 3-MCPD esters) is carried out by using different internal standards. While d_5-3-bromopropane-1,2-diol (d_5-3-MBPD) is used as internal standard for glycidol, d_5-3-chloropropanediol-1,2-bis-palmitol ester is used for the quantification of 3-MCPD originating from 3-MCPD esters. Quantification of glycidol is carried out by the determination of glycidol-induced 3-MBPD and under the application of a transformation factor analogous to Method 1 [2].

Results I: Transformation Factor - Influence of Individual GEs

The quantification of glycidol in Methods 1 and 2 is based on a transformation factor (TF) which is obtained by spiking non-contaminated oil with equidistant concentration levels of one specific GE. The reciprocal value of the slope of the resulting calibration curve equates to the TF [2]. It represents a non-stoichiometric factor reflecting the transformation of glycidol into 3-MCPD (Method 1) and into 3-MBPD in Method 2, respectively. The spiked samples are treated in the same way as the unknown samples. To achieve the calibration line, the use of glycidyl stearate is required. This presupposes that glycidyl stearate is representative of all GEs in the sample - individual GEs have to undergo similar transformations during chemical reactions. Figure 1 illustrates calibration lines and TFs obtained by using different GEs in order to generate 3-MCPD and 3-MBPD, respectively. Table 1 shows glycidol contents calculated when the respective TFs were applied in three different samples.

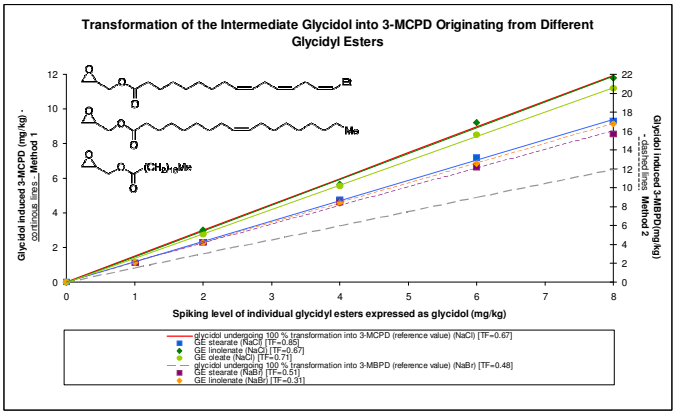

Figure 1: Calibration curves obtained by spiking a blank matrix (flax seed oil) with different concentration levels of GE linolenate, GE oleate and GE stearate. 3-MCPD and 3-MBPD are generated from glycidol under conditions occurring in the course of sample preparation.

Results II: Transformation Factor – Influence of Blank Matrix

Apart from the necessity of having a representative GE to reflect the chemical reaction conditions in the sample, blank matrices must correspond to the sample characteristics (e.g. oil composition, partial acylglycerols, pH, chloride). Calibration curves and resulting TFs obtained by spiking different blank matrices with glycidyl stearate are given in Figure 2.

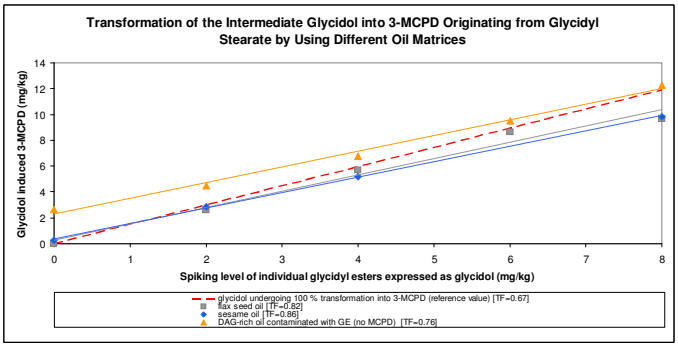

Figure 2: Calibration curves obtained by spiking different oil matrices with concentration levels of glycidyl stearate. The reciprocal value of the resulting calibration curve (slope) equates to the TF which is used for the quantification of glycidol. The application of different TFs to the same sample (3-MCPD Option A: 6.42 mg/kg; 3-MCPD Option B: 3.03 mg/kg) results in the following glycidol calculations (mg/kg): flax seed oil 2.79; sesame oil 2.91; DAG-rich oil 2.58).

Elimination of GE by Acid Treatment of the Sample

Method 3: In Assay 1, the sum of substances that generate 3-MCPD during the analysis (reportedly 3-MCPD esters and GEs) was determined. In parallel (Assay 2), we also determined the level of 3-MCPD resulting from MCPD esters after GEs' elimination by acid treatment of the sample [3]. Assuming that glycidol can be transformed completely into 3-MCPD during sample preparation, the difference between the 3-MCPD contents determined with and without GE elimination is used to calculate the GEs' level on the basis of a stoichiometric conversion factor of 0.67. Results obtained with Method 3 are given in Table 1.

Table 1: Comparison of results obtained by indirect or direct GE determination in three GE-contaminated oil samples

	Indirect Determination Glycidol (mg/kg)								Direct Determination (Total Glycidyl Equiv. in mg/kg)		
	Method 1 [1]			Method [2]			Method 3 [3]		Method 4 [4]		
Sample	*Transformation-factor*	MW n=3	± SD	*Transformation-factor*	MW n=3	± SD	MW n=3	± SD	GE determined*	MW n=3	± SD
DAG-rich Oil	*GE stearate 0.85*	1.47	± 0.02	*GE stearate 0.51*	1.34	±0.03	0.29	± 0.18	GE oleate GE linoleate GE palmitate GE linolenate	1.16	± 0.03
	GE linolenate 0.67	1.15	± 0.01	*GE linolenate 0.32*	0.85	±0.02					
Grape Seed Oil	*GE stearate 0.85*	0.89	± 0.07	*GE stearate 0.51*	0.69	± 0.02	2.07	± 0.06	GE oleate GE linoleate GE palmitate	0.49	± 0.01
	GE linolenate 0.67	0.70	± 0.05	*GE linolenate 0.32*	0.44	± 0.02					
Palm Fat	*GE stearate 0.85*	3.02	± 0.01	*GE stearate 0.51*	2.87	± 0.11	1.51	± 0.29	GE oleate GE linoleate GE palmitate GE linolenate	2.13	± 0.04
	GE linolenate 0.67	2.37	± 0.01	*GE linolenate 0.32*	1.82	± 0.07					

*GE laurate, myristate and stearate were not determined in any sample, GE linolenate was not detected in grape seed oil.

Results III: Direct Determination of GE by LC-MS

A different approach is the direct measurement of single GE by LC-MS. An inherent limitation of direct determinations is the lack of reference compounds; only seven GEs are available at the moment. Due to the absence of appropriate product ions, tandem MS is not more sensitive than single MS. The quantification by single MS of GEs is influenced by the apparent presence of interfering substances in the chromatogram and results in limited specificity. Table 1 compares the results of three GE-contaminated oil samples analysed by direct and indirect determination. Complex chemistry and different approaches are influencing the results of indirect methods. It is an advantage of direct methods that neither transesterification nor glycidol transformation and derivatization are necessary . For the purpose of proper quantification, several reference compounds must be available to prevent underestimation of GE levels. Results may also depend on sample composition.

Conclusions

- The reliability of indirect methods depends on the precondition that all individual GEs have similar transformation rates during the chemical reactions. Different parameters influencing the results were identified.
- In order to achieve the TF, the use of GE linolenate leads to lower glycidol contents than using GE stearate.
- The complexity of indirect approaches due to an inherent complex chemistry is reflected by the illustrations of Figures 1 + 2.
- Prerequisites for indirect methods (like the use of stoichiometric conversion factors) should be scrutinized prior to their application.
- Comparing results achieved by direct and indirect methods, glycidol contents determined using Method 3 deviate to a large extent from results obtained with Methods 1, 2 and 4. In this context, high SDs (up to 62 %) are worth mentioning.
- For the quantification of GEs by direct methods, the availability of standards of the individual GE seems necessary, elsewhere, GEs contents can be underestimated. To exclude inconsistent results, the absence of co-eluting interfering substances and the monitoring of more than one ion would be advantageous. Uncertainty of measurement must be taken into account when calculating a sum of glycidol contents resulting from single ester analysis.

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Literature
[1] DGF Standard Method C-VI 18 (10) Fatty-acid-bound 3-chloropropane-1,2-diol (3-MCPD) and 2,3-epoxipropane-1-ol (glycidol). Determination in oils and fats by GC/MS (Differential measurement)
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