Determination of 3-MCPD, 2-MCPD and glycidyl fatty acid esters in olive oil and waffle using GC-MS/MS method

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Overview
- The determination of 3-MCPDEs, 2-MCPDEs and GEs in processed food is a topical issue since there is only a limited information available.
- A selective analytical GC-MS/MS method for the determination and quantification of 3-MCPD, 2-MCPD and glycidyl fatty acid esters in olive oil and waffle products was developed.
- Several validation parameters were successfully tested within the validation.

Introduction
Fatty acid esters of 3-monochloropropanediol (3-MCPDEs), of 2-monochloropropanediol (2-MCPDEs) and of glycidol (GEs) are formed during the refining of edible fats and oils. They are contaminants of processed edible oils which are used as foods or food ingredients.

3-MCPD is classified as a possible human carcinogen and in 2016 the Joint FAO/WHO Expert Committee on Food Additives decided that glycidol is a genotoxic and carcinogenic compound. Glycidyl esters are significantly hydrolysed to glycidol and elicit toxicity as glycidol. Therefore, the maximum levels (MLs) for glycidol fatty acid esters expressed as glycidol will be set for both vegetable oils and fats and for infant formula (1).

The determination of 3-MCPDEs, 2-MCPDEs and GEs in processed food is a topical issue since there is only a limited information available. In this study, a selective analytical method for the determination and quantification of 3-MCPD, 2-MCPD and glycidyl fatty acid esters in olive oil and waffle products was developed. The indirect gas chromatography-tandem mass spectrometry method was based on the Standard Operating Procedure (SOP) by the EC-JRC-IRMM (2) and the publication by Samaras et al. (2016) (3).

Methods
The validated matrices consisted of extra virgin olive oil and Belgian waffle. Due to the differences in sample matrices the waffle samples were first freeze-dried and extracted using the pressurised liquid extraction (PLE) whereas the olive oil samples were used as such. The flow chart for the analysis of 3-MCPDEs, 2-MCPDEs and GEs with GC-MS/MS technique is shown in Figure 1.

The final detection and quantification of the analytes were performed using a gas chromatograph (Agilent, 6890N) coupled to a Micromass Quattro Micro GC triple quadrupole analyser (Waters, Micromass). Analysis was carried out using the DB-5ms capillary column from Agilent J&W (30 m x 0.25 mm x 0.25 µm). One microliter of the final sample extract was injected in the splitless mode by utilising the electron ionisation (EI) technique (70 eV). Ion transitions were monitored with the multiple reaction monitoring (MRM) mode.

Results
Method validation was done by conducting three replicate analyses at two concentration levels (470 µg/kg and 941 µg/kg) during three different days. Additionally, 12 blank samples were analysed. The following parameters were successfully tested: specificity, selectivity, linearity, repeatability, within-laboratory reproducibility, apparent recovery, the limit of detection (LOD), the limit of quantification (LOQ) and trueness.

The results of within-laboratory reproducibility are illustrated in Table 1. The calculated LOD and LOQ of the method were between 1.9-2.7 µg/kg fat and 3.3-5.7 µg/kg fat in olive oil, respectively. Due to the lack of blank waffle matrix the estimation of LOD and LOQ was not possible at this point. The trueness was assessed by using proficiency test material. Measurement uncertainty (MU) was based on the validation and proficiency test data. MU was estimated as expanded uncertainty, varying from 6.9 to 16% in olive oil and from 10 to 25 % in waffle at concentration level 470 µg/kg.

Conclusions
Based on our validation results we can conclude that the analytical method is suitable for determination of 3-MCPDEs, 2-MCPDEs and GEs in olive oil and waffle products.

References
1. Joint FAO/WHO expert committee on food additives. Eighty-third meeting, Rome 8-17 November 2016. JECFA/83/SC.

Table 1. Within-laboratory reproducibility (RSD%) at the concentration level of 470 µg/kg.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Spiked olive oil (RSD%)</th>
<th>Spiked waffle (RSD%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-MCPD from esters</td>
<td>5,1</td>
<td>12,0</td>
</tr>
<tr>
<td>2-MCPD from esters</td>
<td>3,4</td>
<td>8,0</td>
</tr>
<tr>
<td>3-MBP from glycidyl esters</td>
<td>8,1</td>
<td>5,3</td>
</tr>
</tbody>
</table>

RSD% = relative standard deviation

Figure 1. Flow chart for the analysis of 3-MCPDEs, 2-MCPDEs and GEs.

Figure 2. Multiple reaction monitoring chromatogram of 3-MBPD from GEs in a spiked waffle sample (941 µg/kg).

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