Research on the edible oils contaminants 3-MCPD and glycidol in the framework of the K1 competence center for Feed and Food Quality, Safety and Innovation (FFoQSI)

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Abstract. It is an important issue of FFoQSI to develop, improve and adapt analysis methods that are required in the feed and food industry. The main mission of FFoQSI is to make food and feed production better, safer and more sustainable. A challenging project within the FFoQSI network is the development of a mitigation strategy for the formation of process contaminants during the refining process of edible oils. Precisely, exsisting analysis methods for the process contaminants, 3-chloropropane-1,2-diol (3-MCPD) and glycidol, need to be improved. There are three official methods from the American Oil Chemist´s Society (AOCS), British Standards Institution (BSI) and Deutsche Gesellschaft für Fettwissenschaft (DGF) for the determination of 3-MCPD and glycidol. However, consisting methods are characterized by several drawbacks. For example, some assays are based on the indirect determination of these compounds. In order to reduce analysis time while securing reliability, we suppose to improve consisting methods for simultaneous determination of 3-MCPD and glycidol esters in edible oils.

Keywords: 3-MCPD, glycidol, edible oils.

1 Introduction

The competence center for Feed and Food Quality, Safety and Innovation (FFoQSI) aims to make feed and food production better, safer and more sustainable. To accomplish this mission, relevant steps along the value chains feed – food are examined and the expertise of well-known Austrian research institutions with the know-how of more than 30 innovative enterprises from several countries is combined. A challenging project within FFoQSI is the development of a mitigation strategy for the formation of process contaminants during the refining process of edible oils, namely 3-chloropropane-1,2-diol (3-MCPD) and glycidol. 3-MCPD was first discovered as a process

contaminant in acid-hydrolyzed vegetable protein by Devídek *et al.* in 1978 and from there on detected in many kinds of processed foodstuff and also identified as 3-MCPD fatty acid ester in refined vegetable oils by Zelinková *et al.* in 2006 [1], [2]. Due to the process of method development for 3-MCPD analysis later on also glycidol fatty acid esters were found in edible oils and declared as "3-MCPD related compound" [3]. While factors for 3-MCPD ester formation are not completely known yet, formation of glycidol ester is mainly dependent on temperature and dwell time during the refining process [4]. However, there is still a lack of knowledge of the influencing variables for the formation of 3-MCPD esters, but both contaminants are highly estimated to be carcinogenic and hephrotoxic by the Bundesinstitut für Risikobewertung. Nevertheless, the challenge to detect 3-MCPD and glycidol esters yielded in several analytical methods ranging from direct detection by LC ToF MS to indirect detection via GC-MS [5]–[9]. In comparison to indirect GC-MS methods, the sample preparation for LC-MS methods as well as the interpretation of obtained data are very time consuming. This fact is due to the detection of 3-MCPD- and glycidol fatty acid esters and the big variety of possible fatty acid distribution in oil samples. In contrast GC-MS methods need a previous hydrolysis step to release the process contaminants from fatty acids and make them accessible for further derivatization reactions. In this case the application of a stable isotope internal standard is necessary to determine a transformation factor for further calculation as 3-MCPD and glycidol are not stable under acidic or alkaline conditions. While 3-MCPD is converted to glycidol under alkaline conditions, glycidol reacts to a variety of substances depending on the used acid, present matrix components as well as other ions. The applied GC-MS methods recommended by AOCS, BSI and DGF are based on the simultaneous preparation of a sample in two assays. The first step is a hydrolysis of each assay with methanolic NaOH or sodium methoxide solution, followed by treatment with acidic salt solutions. For one assay, an acidic NaCl solution is used, while the other one is mixed with a chloride free salt solution. During this treatment glycidol is converted to 3-MCPD in the first approach and to the corresponding derivate depending on the used salt in the second approach. After defatting with *iso*-hexane, an extraction with a mixture of diethyl ether and ethyl acetate is carried out previously to a further derivatization with phenylboronic acid (PBA). Approaches are analyzed by GC-MS and the contents of 3-MCPD are determined. The content of glycidol is calculated from the difference of the 3-MCPD contents of both assays and with inclusion of the transformation factor [7]–[9]. The aim of the study is to develop an advanced method for simultaneous analysis of 3-MCPD and glycidol in only one approach. Glycidol is converted to 3-bromo-propane-1,2-diol and can be quantified by usage of a reference standard. Nevertheless, the use of d_5-3-MCPD as internal standard remains necessary as conversion of 3-MCPD during alkaline hydrolysis is inevitable.

2 Material and methods

2.1 Reagents and materials

Sodium hydroxide, sodium sulfate (water free), sodium bromide, phenylboronic

acid, sulfuric acid were obtained from Merck; methanol, *tert*-butyl methyl ether (*t*BME), *iso*-hexane, diethyl ether, ethyl acetate, *iso*-octane (all GC-MS grade) were obtained from ChemLab; nitrogen 5.0 (Air Liquide); 3-chloropropane-1,2-diol, 3-bromopropane-1,2-diol (3-MBPD) and d_5-3-chloropropane-1,2-diol as internal standard were obtained from Sigma-Aldrich. The alkaline hydrolysis reagent is prepared with 20 g L^{-1} NaOH in methanol; the acidic salt solution is prepared with 600 g L^{-1} NaBr and 35 mL L^{-1} H_2SO_4 (25%); the derivatization reagent is prepared as saturated phenylboronic acid solution in diethyl ether, the extraction solution is a mixture of diethyl ether and ethyl acetate (60/40 (v/v)). The external standards are dissolved in the extraction solution, the internal standard is dissolved in *t*BME. Oil samples were provided by VFI (Wels, AT).

2.2 Preparation of samples

100 mg (±0.5 mg) oil sample were weighed into a 2 mL PP-reaction tube and dissolved in 100 µL *t*BME and 100 µL internal standard was added. Alkaline hydrolysis was carried out by addition of 200 µL hydrolysis reagent at room temperature for 4 minutes. Hydrolysis reaction was stopped by addition of 600 µL acidic salt solution. The approach was defatted by addition of 600 µL *iso*-hexane and vortexing for 30 seconds. The organic phase was discarded. Extraction was carried out three times with each 600 µL extraction solution, whereat organic phases were collected in a new 2 mL PP-reaction tube and dried over Na_2SO_4. Derivatization reaction is done by addition of 25 µL saturated PBA solution and vortexing for 60 seconds. The reaction mixture was dried under a nitrogen stream and re-dissolved in 500 µL *iso*-octane. 300 µL of the solution were transferred into a micro-vial and used for GC-MS analysis.

2.3 Analysis of samples by GC-MS

A Trace 1300 gas chromatograph with PTV injector and a RSH TriPlus autosampler coupled to an ISQ QD single quadrupole mass spectrometer (Thermo Fisher) managed by Chromeleon 7.2 was used for analysis. At it, separation was achieved with a TG-5SILMS column (ID 0.25 mm, 0.25 µm film thickness, 30 m; Thermo Fisher) and helium 5.0 at a constant flow of 2 mL min^{-1} as carrier gas. The initial temperature for gas chromatography was 85 °C with a hold time of 0.5 min. The temperature was increased first to 150 °C with a heating rate of 6 °C min^{-1}, then with 12 °C min^{-1} to 180 °C and 25 °C min^{-1} to 280 °C. The total run time was 25 minutes. Injection temperature of the PTV injector was set to 85 °C and a split ratio of 1:100 was hold for 0.5 min as evaporation phase. To transfer the analytes to the chromatographic system, the PTV was heated up to 150 °C with 6 °C s^{-1} at splitless mode. As a cleaning step the PTV was further heated to 400 °C at a rate of 14 °C s^{-1} for 8 min with a split ratio of 1:100. The injection volume was 8 µL. The mass spectrometer transfer line temperature was set to 280 °C, ion source temperature was set to 230 °C. Detection was performed in SIM mode at m/z 147, 149, 196, 201 and 240, whereat 147

was used as quantifier ion for 3-MCPD and 3-MBPD, 196 and 240 were used as qualifier ions. For d_5-3-MCPD m/z 149 was used as quantifier ion and 201 was used as qualifier ion.

2.4 Calculation of 3-MCPD and glycidol

For the calculation of the concentration of 3-MCPD in the approach, at first the transformation factor *t* has to be calculated. Therefore, the concentration of applied internal standard (ISTD) is divided by the measured concentration of internal standard (equation 1).

$$t = \frac{applied\ conc.ISTD}{measured\ conc.ISTD} \tag{1}$$

Consequently, the measured concentration of 3-MCPD has to be multiplied by the transformation factor *t* as shown in equation 2 below.

$$c_{3-MCPD} = measured\ conc.\ 3-MCPD * t \tag{2}$$

For the calculation of the concentration of glycidol the measured concentration of 3-MBPD has to be divided by the transformation factor *t* and further multiplied by the molar factor *f*, that represents the ratio of molar masses of glycidol and 3-MBPD (equations 3 and 4).

$$c_{Glycidol} = \frac{measured\ conc.\ 3-MBPD}{t} * f \tag{3}$$

$$f = \frac{M_{Glycidol}}{M_{3-MBPD}} = \frac{74.08\frac{g}{mol}}{154.99\frac{g}{mol}} = 0.4779 \tag{4}$$

Obtained concentrations of 3-MCPD and glycidol have to be regarded to the sample weight to achieve the mass fractions of the contaminants

3 Results and discussion

3.1 Results

The desired advancement of consisting analysis methods for the detection of 3-MCPD and glycidol esters by GC-MS resulted in the successful detection of both analytes using a strategy shown in Fig. 1. It was achieved to detect 0.6 mg kg^{-1} 3-MCPD and 0.3 mg kg^{-1} glycidol in a tested sunflower oil sample. The conversion to suitable compounds for GC-MS analysis occurs as follows. Alkaline hydrolysis (A) results in liberated 3-MCPD and glycidol (B). The obtained 3-MCPD remains intact, while free

glycidol undergoes a reaction to 3-bromopropane-1,2-diol after addition of an acidic NaBr solution (C). Both intermediates further react with PBA (D) and gain the necessary volatility for GC-MS analysis.

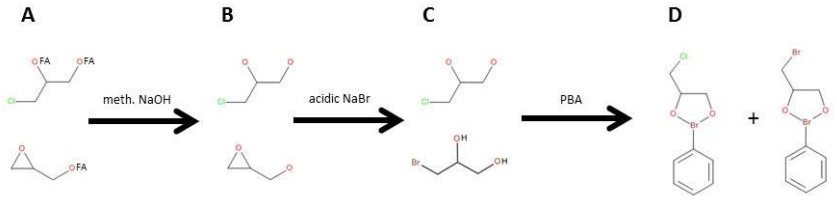

Fig. 1. Reaction scheme of 3-MCPD and glycidol esters during sample preparation for GC-MS analysis.

3.2 Discussion

The chosen basic experimental procedure for the detection of 3-MCPD and glycidol esters by GC-MS appears appropriate. However, single preparation and reaction steps need to be improved and adapted. As the method includes a washing step, an extraction step and three reaction steps it is highly sensitive to any kind of variation. In this context, simplification to a single step procedure would be a significant benefit. The FFoQSI competence center provides optimal frame conditions for addressing these important questions.

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