Journal of Chromatography A, 1466 (2016) 136–147

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Analytical method for the trace determination of esterified 3- and 2-monochloropropanediol and glycidyl fatty acid esters in various food matrices

Vasilios G. Samaras, Anupam Giri, Zuzana Zelinkova, Lubomir Karasek, Gerhard Buttinger, Thomas Wenzl*

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Retieseweg 111, 2440 Geel, Belgium

ARTICLE INFO

Article history:
Received 8 June 2016
Received in revised form 30 August 2016
Accepted 30 August 2016
Available online 31 August 2016

Keywords:
3-MCPD esters
2-MCPD esters
Glycidyl esters
Food
Process contaminants

ABSTRACT

Fatty acid esters of 3-monochloro-1,2-propanediol (3-MCPDEs), of 2-monochloro-1,3-propanediol (2-MCPDEs) and of 2,3-epoxy-1-propanol or glycidol (GEs), which are considered to be deleterious to human health, may occur in a broad variety of food samples. A proper risk assessment of those substances requires the availability of robust occurrence data; in this respect concerns have been raised regarding the reliability of results obtained with the currently available methods to determine those substances in processed food. This article presents an indirect analytical procedure for the simultaneous determination of 3-MCPDEs, 2-MCPDEs and GEs in a wide variety of food products after extraction by pressurised liquid extraction (PLE) and determination by gas chromatography mass-spectrometry (GC–MS). For the differentiation of MCPDEs and GEs, the latter were first converted to monobromopropanediol esters (MBPDEs) in acid aqueous solution of sodium bromide. MCPDEs and MBPDEs were then hydrolysed under acidic conditions followed by derivatisation of the released free (non-esterified) form in ethyl acetate with phenyl boronic acid (PBA). Quantification of the analytes was carried out using the isotopic labelled analogues of both MCPDEs and GEs. Limits of detection (LODs) and limits of quantitation (LOQs) were in the range of 7–17 mg kg^{-1} and 13–31 mg kg^{-1} respectively, while the working range of the method was between LOQ and 1850 mg kg^{-1} expressed on fat basis. The developed method was successfully applied for the analysis of the target compounds in more than 650 different food samples covering the following commodities: bread and rolls, fine bakery wares, smoked fish products, fried and roasted meat, potato based snacks and fried potato products, cereal-based snacks and margarines.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

The increasing concerns about possible health risks due to the presence of fatty acid esters of 3-monochloro-1,2-propanediol and 2-monochloro-1,3-propanediol (MCPDEs) as well as of 2,3-epoxy-1-propanol or glycidol (GEs) in processed edible oils and fats initiated a series of actions by the European Commission (EC), the EU Member States, but also academia, and industry [1,2]. However, high levels of MCPDEs have been reported in refined palm oil [3,4] as well as in infant formula [5], human breast milk [6] and various heat processed foods [7]. Even though research on possible toxic effects and exposure of humans to these substances has not been finalized yet, concerns regarding the presence of these substances in food seem to be justified due to the confirmed release of the MCPD/glycidol moiety from the parent esters in the human intestine [8–11]. The International Agency for Research on Cancer (IARC) classified glycidol as probably carcinogenic to humans (group 2A), and considered 3-MCPD as a possible human carcinogen (group 2B) [12,13]. The Scientific Committee on Food (SCF) of the European Commission established a tolerable daily intake (TDI) of 2 mg kg^{-1} body weight [14], which agrees with the provisional maximum tolerable daily intake (PMTDI) specified by the FAO/WHO Expert Committee on Food Additives (JECFA) [15,16].

In response to that urgency, several analytical methods have been reported for the determination of MCPDEs and GEs in edible fats and oils, as these matrices provide the major source for exposure to MCPDEs and GEs. The analytical methods published so far follow two main routes – the determination of the MCPD and glyci-

* Corresponding author.
E-mail address: Thomas.Wenzl@ec.europa.eu (T. Wenzl).

http://dx.doi.org/10.1016/j.chroma.2016.08.071
0021-9673/© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

dol moieties after hydrolysis of the parent esters and derivatisation of free MCPD and glycidol, and, less frequently, the determination of intact MCPDEs and GEs. A comprehensive overview covering aspects of different analytical approaches was recently published by Crews et al. [17]. Direct analysis of the intact esters glycidyl laurate, myristate, palmitate, stearate, oleate, linoleate, and linolenate has been successfully applied for the analysis of GEs in edible oil [18–20]. However, the determination of the individual MCPDEs is limited due to the large number of possible MCPD mono- and di- esters [18]. Furthermore, these procedures require advanced analytical instrumentation, contributing highly to the total cost of analysis [21]. In contrast, indirect analytical methods involve the conversion of all native mono- and di- fatty acid esters of MCPD and of glycidol into the free forms. Stabilization of glycidol by conversion of the epoxide into less reactive bromo- or methoxy-derivatives of glycerol may be performed prior or after ester hydrolysis [7,22,23]. Hydrolysis may be conducted under alkaline or acidic conditions [7,22,23]. Derivatisation of free MCPD and monobromopropanediol (MBPD) or methoxypropanediol (MPD) respectively, is finally followed by gas chromatographic-mass spectrometric (GC–MS) measurement. Enzymatic hydrolysis of the esters has also been used [24–26].

Indirect analytical methods are favored for routine analysis of MCPDEs and GEs, despite issues regarding the likely mutual interconversion/transformation of 3-MCPD esters and GEs and the broad range of left-censoring limits (LC limit) among the methods [27].

A method comparison study, organized in 2012 by the Joint Research Centre (JRC), on the determination of 3-MCPDEs and GEs in edible oils confirmed the comparability of results obtained after acid catalyzed and alkaline catalyzed hydrolysis of the esters, respectively [28]. However, a recent study performed by Weisshaar, as well as the study of the JRC highlighted that artefact formation of the two groups of compounds during analysis might cause bias [3]. The need of harmonized analytical methodology for the determination of MCPDEs and GEs was satisfied only for edible oils where three indirect analytical methods were validated by collaborative trial [29–31]. Additionally, one direct analytical method was validated for the determination of GEs in edible oils [18]. The procedures were adopted by the American Oil Chemists' Society (AOCS) as AOCS standard methods.

In 2013, the Scientific Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority (EFSA) issued a Scientific Report emphasizing the need of a standardized method to reduce the uncertainty in occurrence and exposure estimates of MCPDEs and GEs in food [32]. The accurate determination of these substances in food matrices remains a challenge due to the low limits of quantitation (LOQs) required by the regulatory bodies, the composition of the sample matrix and the varying fat content to be dealt with [1]. Commonly, most methodologies used for determining MCPDs and GEs comprise the isolation of fat from the food sample followed by subsampling of the fat fraction for further analysis. Some information on the performance of certain analytical methods can be extracted from a comparative study on the determination of 3- and 2-MCPDEs in infant formula, mayonnaise, and vegetable oil based creams and spreads, which was organized in 2011 by the German Federal Institute for Risk Assessment (BfR) [33]. The BfR study demonstrated that pressurized liquid extraction (PLE) comprising several extraction cycles provided complete fat extraction and high levels of analyte recovery, superior to conventional extraction techniques (e.g. sonication, Soxhlet, stirring, shaking) [33]. Besides this report only few papers report on the performance of analytical methods for the determination of 3-MCPDEs in food [2,5,6,34–36]. Less is known about the performance of methods that allow besides the determination of 3-MCPDEs the simultaneous determination of 2-MCPDEs and GEs. Küsters et al. [7,37] presented method performance data for the analysis of 3-MCPDEs and GEs in a broad range of food, with large differences in composition. However, the accuracy of the method used was questioned by a recent study [2] due to the potential partial transformation of GEs to 3-MCPD during sample preparation in alkaline media leading to bias. Karl et al. [34] modified an analytical method standardized by AOCS in order to determine 3- and 2-MCPDEs and GEs in fishery products. Next to the determination of the esters, this method also allows the determination of free 3-, 2-MCPD from the same test portion extract.

Controversy exists on the contribution of food processing to the generation of MCPDEs. Generation of MCPDEs was identified in the frame of the EU funded project PROMETHEUS during baking of cookies [38]. However, this finding was questioned, since significant generation of MCPDEs and GEs was not observed in deep-fried potatoes [39].

Occurrence data on MCPDEs and GEs in thermally treated products other than fats and oils are scarce with limited availability of data published in peer reviewed journals [5–7,34,35].

In the context of a preliminary assessment of human exposure to MCPDEs and GEs in thermally treated food commodities, EFSA requested the Joint Research Centre (JRC) a Directorate General of the European Commission to develop and validate an indirect analytical method for determining 3-MCPDEs, 2-MCPDEs, and GEs in a wide variety of food matrices with adequate performance parameters, in particular LC limits. Methods recently standardized by AOCS for the determination of MCPDEs and GEs in edible oils and fats had to be used as a basis for method development and optimization. For practical reasons, the method that includes acidic transesterification of the ester forms [29] was used as a building block for the design of the analytical method described here. Due to the limited scope of the analytical methods standardized by AOCS, it was necessary to demonstrate the reliability of the data produced by the analytical method proposed in this manuscript. Carefully assessed were in this respect fat extraction yield, precision, accuracy and sensitivity of the developed method. The stability of performance of the analytical method was monitored via plotting of results of QC samples in Shewhart control charts.

2. Materials and methods

2.1. Analytical standards and reagents

High purity analytical standards (>98.5%) of *rac* 1,2-bis-palmitoyl-3-chloropropanediol (3-MCPD), *rac* 1,2-bis-palmitoyl-3-chloropropanediol-D_5 (3-MCPD-D_5), 1,3-distearoyl-2-chloropropanediol (2-MCPD), 1,3-distearoyl-2-chloropropanediol-D_5 (2-MCPD-D_5), glycidyl palmitate (Gly-P) and glycidyl oleate-D_5 (Gly-O-D_5) were supplied by Toronto Research Chemicals Inc (Toronto, Canada). Individual stock solutions (1000 $\mu g\,mL^{-1}$) of native and isotope labelled MCPD esters and GEs were prepared by dissolving 10 mg of each analyte in 10 mL of toluene and stored at 4 °C. The expiration date of the stock standard solutions was six months. These solutions were then mixed together and diluted with toluene to obtain a solution of intermediate concentration at 5.4 $\mu g\,mL^{-1}$, which was stored at 4 °C in the dark for no more than one month. 50 μL of the mixed stable isotope labelled esters solution (5.4 $\mu g\,mL^{-1}$) was used for the spiking into the fat extract and for calibration (for internal standardisation).

CAS registry numbers, molecular formulas and molecular weights of the target compounds are given in Table 1.

Anhydrous tetrahydrofuran, methanol, isohexane, *n*-hexane, ethyl acetate, 2,2,4-trimethylpentane (isooctane), diethyl ether, toluene and *tert*-butyl methyl ether (t-BME) were of HPLC grade purchased from either Sigma-Aldrich (Bornem, Belgium) or VWR

Table 1
CAS number, molecular formula and molecular weight of native and labelled analytes.

Name	Acronym	CAS #	Molecular formula	Molecular weight ester	Molecular weight free form	R_M [a]
rac 1,2-bis-palmitoyl-3-chloropropanediol	3-MCPD ester	51930−97-3	$C_{35}H_{67}ClO_4$	587.36	110.54	0.1882
rac 1,2-bis-palmitoyl-3-chloropropanediol-D_5	3-MCPD-D_5 ester	1185057−55-9	$C_{35}H_{62}D_5ClO_4$	592.39	115.57	0.1951
1,3-distearoyl-2-chloropropanediol	2-MCPD ester	26787−56-4	$C_{39}H_{75}ClO_4$	643.46	110.54	0.1718
1,3-distearoyl-2-chloropropanediol-D_5	2-MCPD-D_5 ester	–	$C_{39}H_{75}ClO_4$	648.49	115.57	0.1782
glycidyl palmitate	Gly-P	7501−44-2	$C_{19}H_{36}O_3$	312.48	74.08	0.2371
glycidyl oleate-D_5	Gly-O-D_5	5431-33-4-unlabelled	$C_{21}H_{33}D_5O_3$	343.56	74.08	0.2156

[a] R_M: Ratio of mol weights between the free forms of MCPD and glycidol, and the respective MCPD and glycidyl esters.

(Leuven, Belgium). Phenylboronic acid (≥97%), ultra-pure sulphuric acid (≥95%), sodium hydrogen carbonate (≥99%), anhydrous granular sodium sulphate, (≥99%), ammonium sulphate (≥99%), anhydrous sodium bromide (≥99.5%) were purchased from Sigma-Aldrich and Merck (Darmstadt, Germany), respectively. Sodium polyacrylate and sand (50–70 mesh particle size) were obtained from Sigma-Aldrich. Ultrapure water was obtained in-house with a Milli-Q water purification system (Millipore, Bedford, MA, USA).

The acid aqueous solution of sodium bromide (3 mg mL^{-1}) was prepared by mixing 180 μL of a concentrated aqueous solution of sodium bromide (1 g of sodium bromide in 10 mL of ultra-pure water) with 5.5 mL of ultra-pure water and 0.3 mL of sulphuric acid into a 10 mL volumetric flask.

The sodium hydrogen carbonate saturated solution and ammonium sulphate solution were prepared by mixing 4.8 g of $NaHCO_3$ and 20 g of $(NH_4)_2SO_4$ in 50 mL of ultra-pure water, respectively.

The sulphuric acid/methanol solution used in the acid catalyzed transesterification reaction was prepared in a 100 mL volumetric flask by addition of 1.8 mL of concentrated sulphuric acid to 50 mL methanol and filling then with methanol up to volume.

2.2. Sampling

Food samples covered seven food categories including bread and rolls, fine bakery wares, smoked fish products, fried and roasted meat, potato based snacks and fried potato products, cereal-based snacks and margarines. Sampling was performed in retail stores, restaurants and from different households in Europe covering countries such as Austria, Belgium, Czech Republic, Denmark, France, Italy, Germany, Greece, Latvia, Portugal, Spain, The Netherlands and the United Kingdom. Samples were stored according to the manufacturer's recommendations and analysed within the expiration date after their collection.

For method development and validation experiments, an extra virgin olive oil blank sample was obtained from a local retail market in Greece. The material was stored in the dark at room temperature.

2.3. Sample pretreatment and PLE

To ensure good extraction efficiency, portions of the solid test samples were immersed in liquid nitrogen and then grinded and homogenized, by means of a laboratory grinder, or mortar and pestle, to fine homogeneous powders. Test portions of 5 g were mixed with 5 g of polyacrylate and 15 g of sand and transferred into the pressurized liquid extraction (PLE) cell. PLEs of the target compounds from food samples were carried out using an ASE™ 300 extractor (Dionex™, Thermo Scientific™, Sunnyvale, CA, USA) equipped with 33 mL stainless steel extraction cells. The extraction parameters were as follows: oven temperature: 40 °C, pressure: 103 bar, pre-heating time 1 min, static extraction time 5 min, heating 5 min, two static cycles, flush volume 100% of cell volume, purge time with N_2 for 60 s.

The extraction solvents tested in this study were based on extractants, which were proposed in literature for the determination of MCPDEs and GEs in infant formula (a mixture of petroleum ether/iso-hexane/acetone (P/I/Ac) = 2/2/1 *v*/*v*, PLE at 125 °C) [33], in different other food commodities (t-BME at room temperature) [7], and in margarine (t-BME/*n*-heptane 2/1 *v*/*v* at 80 °C) [2]. Based on the outcome of these tests, 100% t-BME at 40 °C was applied for all further extractions.

To overcome the influence of the great spread of fat contents of the test samples, internal standards were added prior to or after the fat extraction, based on the expected fat content. For breads and rolls, porridge and other food matrices with labelled total fat contents below or equal to 5% (weight/weight), stable isotope labelled internal standards were added directly into the PLE extraction cell containing the test portion prior to sample extraction. If the total fat content of the test sample exceeded the 5% threshold, stable isotope labelled internal standards were added after the extraction to the 100 mg portion of fat/oil that was used for further sample preparation.

The obtained extract (∼100 mL) was decanted into an evaporation vessel with known tare weight and evaporated with a vacuum evaporator (Büchi Rotavapor R-114 a Waterbath B-480, Darmstadt, Germany) at 40 °C until dryness. The extracted amount of fat was then determined gravimetrically. Consequently, a portion of 100 mg of fat/oil (±5 mg) of the remaining fat fraction was transferred with a Pasteur pipette or a spatula into a 10 mL screw cap glass tube along with 2 mL of anhydrous tetrahydrofuran for reconstitution.

The whole residue was further cleaned up in case of test samples with an expected fat content level below 5% (w/w).

Margarines, consisting mostly of lipids, were directly spiked with stable isotope labelled internal standards and vortex-mixed with anhydrous tetrahydrofuran.

2.4. Glycidyl ester conversion and acid transesterification

For glycidyl ester conversion into monobromopropane diol esters, 30 μL of aqueous sulphuric acid solution of sodium bromide (3 mg mL^{-1}) were added to the sample, shaken vigorously (vortex) and incubated at 50 °C for 15 min. The reaction was stopped by the addition of 3.0 mL of 0.6% aqueous solution of sodium hydrogen carbonate. To separate the oil/fat from the water phase, 2.0 mL of *n*-hexane were added and vigorously mixed on a vortex mixer. After separation of the two phases, the upper layer was transferred to an empty test tube and evaporated at 40 °C to dryness under a gentle stream of nitrogen. Finally, the residue was dissolved in 1.0 mL of anhydrous tetrahydrofuran.

Acid transesterification was performed with the addition of 1.8 mL sulphuric acid/methanol solution to the sample and shaken vigorously for 10 s. The mixture was incubated at 40 °C for 16 h. After the incubation period, the ester cleavage reaction was stopped by the addition of 0.5 mL of a saturated solution of sodium hydrogen carbonate to the sample. Evaporation of the organic solvent (methanol) from the mixture was performed at 40 °C under a nitrogen stream.

Afterwards, 1.3 mL of ammonium sulphate solution was added to achieve a salting-out effect in the consequent extraction. Fatty acid methyl esters were separated from the sample by two consecutive liquid–liquid extractions each with 1.0 mL *n*-hexane. Finally, extraction of the released (free) forms of 2- and 3-MCPD as well as 3-MBPD from the aqueous phase was accomplished three times with 0.6 mL of ethyl acetate. The upper phases were transferred to an empty glass test tube containing a small amount of anhydrous granular sodium sulphate.

2.5. Derivatisation procedure

Derivatisation of the analytes was performed with phenyl boronic acid (PBA) in organic solution instead of aqueous medium as described in the AOCS method [29]. The benefit of this step was lower consumption of derivatisation reagent, which provided lower background levels, and consequently lower LODs. In particular, derivatisation was performed by adding 150 μL of the derivatisation reagent (0.4 g of phenylboronic acid in 10 mL of diethyl ether) to the combined ethyl acetate extract. The solution was then vortex-mixed for 15 s and incubated in an ultrasonic bath for 5 min. To complete the derivatisation reaction, the extract was evaporated at 40 °C to dryness under a low stream of nitrogen. The residue was dissolved in 300 μL of isooctane by shaking the mixture for 10 s (vortex) and finally centrifuged at 3500 rpm. The supernatant was transferred for gas chromatographic measurement to an empty GC vial with a glass insert of about 150 μL of volume. Fig. 1 illustrates the analytical scheme applied for the determination of MCPD esters and GEs in food.

2.6. Gas chromatography-mass spectrometry analysis

Gas chromatographic analysis was carried out with a Hewlett Packard gas chromatograph 5890 Series II. The capillary column was a DB5MS (30 m × 0.25 μm × 0.25 mm film thickness) from Agilent Technologies (Santa Clara, USA). Samples were injected (1.0 μL) into the GC in pulsed splitless mode at 280 °C. The carrier gas was helium (99.999%) set at a constant flow of 1.2 mL min^{-1}. For chromatographic separation of all derivatives, the temperature was programmed as follows: 60 °C for 1.0 min, from 60 °C to 150 °C at 6 °C min^{-1}, 2.0 min at 150 °C and from 150 °C to 300 °C at 10 °C min^{-1}. The gas chromatograph was connected to a Hewlett Packard Mass Spectrometer HP5971 MSD (Palo Alto, CA, USA) operated in electron ionisation (EI) mode at 70 eV ionization energy. The MS transfer line temperature was maintained at 300 °C, whereas the ion source and quadrupole temperatures were 250 °C and 150 °C, respectively. Full-scan mass spectra of the derivatives were obtained over a range of *m*/*z* 50–400. Chromatograms were recorded in selected ion monitoring (SIM) mode. For each compound, the most abundant ions were selected for quantitation except for 3-MBPD due to potential interferences. Detailed information of the elution order, retention times and characteristic ions used for GC–MS analysis are shown in Table 2.

Table 2
Retention times and *m*/*z*-ratios of native and stable isotope labelled MCPDs and MBPD for GC–MS (SIM) analysis.

Compound	Retention time (min)	Quantification ion Q_1 (*m*/*z*)	Confirmation ion Q_2 (*m*/*z*)
3-MCPD-D_5	16.87	150	201
3-MCPD	16.95	147	196
2-MCPD-D_5	17.61	201	203
2-MCPD	17.70	196	198
3-MBPD-D_5	18.90	150	245
3-MBPD	18.96	146	240

2.7. Calculations

For the preparation of calibration standards, the actual concentrations were calculated for each standard/analyte applying Eq. (1).

$$M_{cal} = C_I * V_I * R_M \tag{1}$$

Where:

M_{cal} Equivalent of free form of native (labelled) substance in test tube (in ng)

C_I Concentration of intermediate standard solution used to prepare calibration standard (in μg mL^{-1})

V_I Volume of intermediate standard solution pipetted into test tube (in μL)

R_M Ratio of mol masses of native (labelled) free forms of 3-MCPD, 2-MCPD respectively glycidol and the corresponding esters used to prepare calibration standards.

Instrument calibration was performed with standards of the respective esters 3-MCPD 2-MCPD, 3-MBPD, as well as their stable isotope labelled analogues. They were mixed with extra virgin olive oil and subjected to the whole analysis procedure, starting with the bromination of glycidyl esters, followed by acid transesterification and finally derivatisation. The derivatives were at the end extracted into isooctane and analysed by GC–MS. The calibration curves were obtained by plotting the signal ratios of the PBA derivatives of the native analytes and the PBA derivatives of the corresponding labelled standards on the abscissa, against the amounts of native analytes (expressed in ng of free 3-MCPD, free 2-MCPD, or glycidol equivalents, respectively) added into the test tube prior to derivatisation. The calibration functions were defined for each analyte by linear regression.

The concentration of the esterified form of analytes in the analysed fat/test portion was expressed in μg kg^{-1} according to Eq. (2).

$$X_{native} = \frac{\frac{\left(\frac{A_{native}}{A_{labelled}} - b\right)}{a}}{W_{sample/fat}} \tag{2}$$

X_{native} is the concentration of native analytes (in μg kg^{-1}) in the analysed fat/test sample.

A_{native} is the area of the native analyte peak of the test sample

$A_{labelled}$ is the area of the corresponding stable isotope labelled analyte peak

α is the slope of the calibration function

b is the intercept of the calibration function

$W_{sample/fat}$: a) for food matrices with total fat content > 5% (w/w): weight of the extracted fat used for further analysis, or b) In cases where the expected labelled total fat content of the test sample was below or equal to 5% (weight/weight): weight of test portion; (both values in g)

Eq. (2) provides for test samples with total fat content levels above 5% (w/w) the result on fat basis. They were then converted into the analyte content value expressed on product basis (μg kg^{-1}) using the determined fat contents according to Eq. (3):

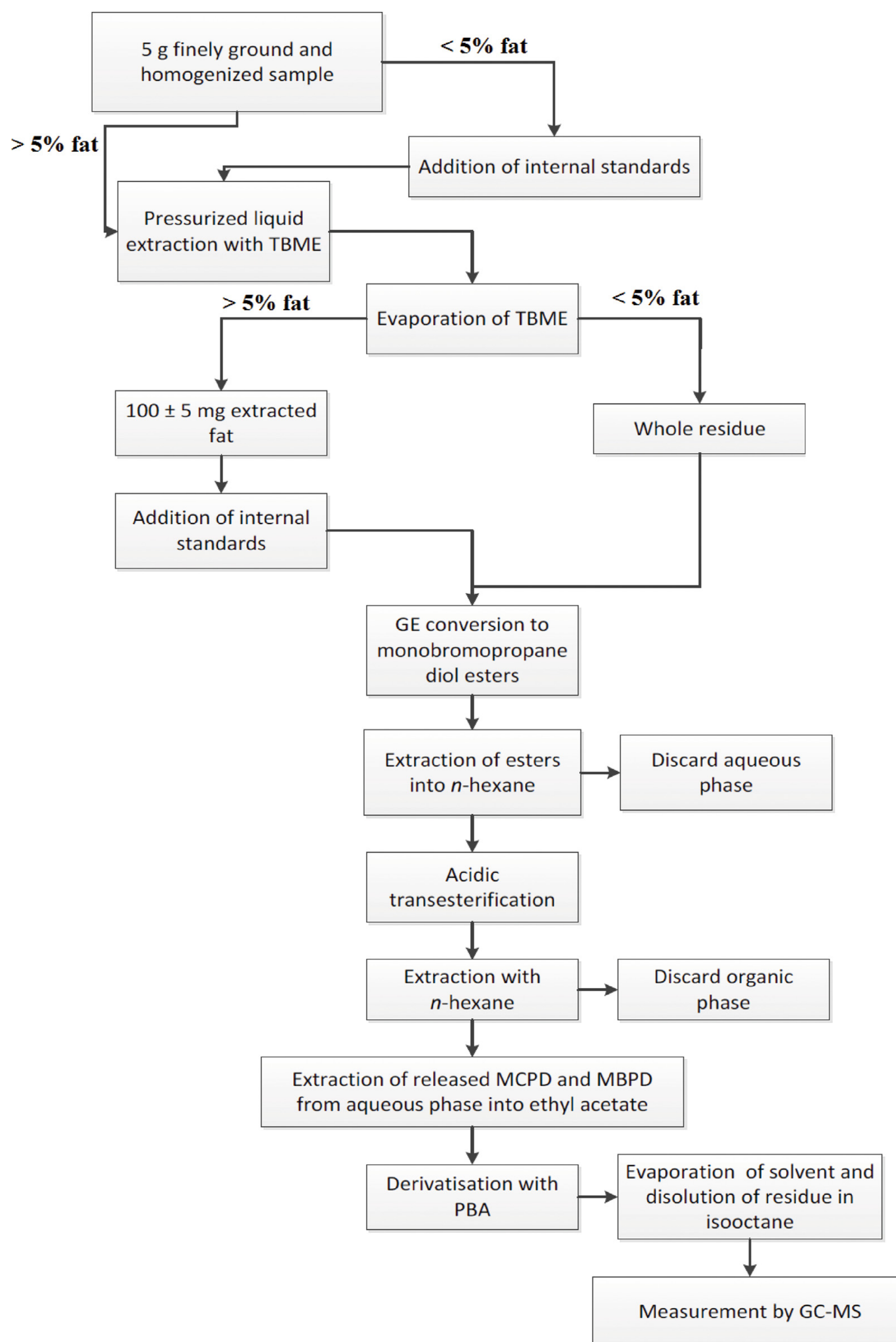

Fig. 1. Analytical scheme for the determination of 3-MCPD esters, 2-MCPD esters and GEs in food.

This approach had the benefit of providing simultaneously results on fat basis and on product basis.

$$C_p = X_{native} * \frac{F_{extracted}}{W_{sample}} \quad (3)$$

C_p concentration of the native compound in the sample (**in μg kg^{-1}**)

X_{native}: is the concentration of native analytes (in μg kg^{-1}) in the analysed fat/test sample.

$F_{extracted}$; Amount of fat extracted from the test portion (**in g**)

W_{sample}; Weight of the test portion (**in g**)

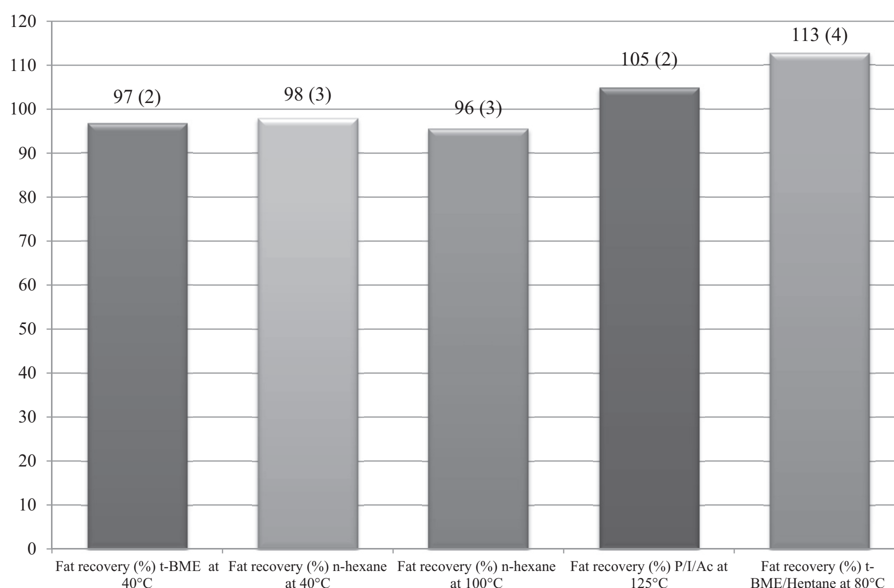

Fig. 2. Comparison of different extraction protocols based on Pressurized Liquid Extraction (PLE) technique for complete fat recovery from a waffle sample (expressed in percentage).

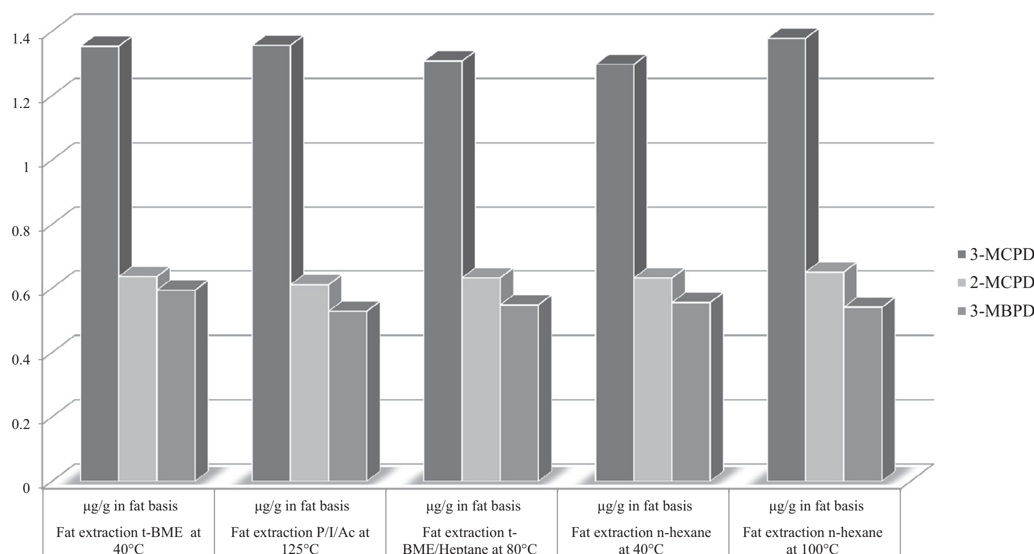

Fig. 3. Analyte content measured in extracts (expressed in $\mu g\,g^{-1}$ on fat basis) obtained from a Belgian waffle sample with different extraction protocols.

3. Results and discussion

3.1. Optimization of the extraction procedure

The extraction of both fat and analytes from food samples by means of PLE was investigated and optimized. The optimization of the extraction procedure included the type of organic solvent and the extraction temperature. The setting of extraction parameters was based on previous studies [2,7,33] and experiences made in other areas, which also require the extraction of fat from food. The standard fat extraction method according to Weibull-Stoldt was avoided due to the harsh conditions, leading potentially to artefact formation.

Fig. 2 depicts the fat extraction yields from a Belgian waffle sample for the studied extraction protocols. This sample was characterised by a labelled fat content of 22.2 g fat per 100 g product. According to the results, all different extraction solvents provided extraction yields agreeing with each other within 10% and with the labelled total fat content within 5%. The extraction with t-BME/*n*-heptane resulted in exceeding the labelled total fat content by 8.3% to 17.9%. This bias in the fat determination might be attributed to incomplete evaporation of *n*-heptane, which is difficult to evaporate at moderate evaporation conditions (40 °C). Furthermore, the analyte contents were determined in the extracts obtained with the different extraction protocols. Results were expressed both on fat (Fig. 3) and on product basis (Fig. 4). All experiments were conducted in triplicate. The outcome indicates a high degree of comparability of results under the applied extraction conditions.

Fat extractions experiments were also performed with other food matrices in order to assess a potential matrix dependency. Fig. S1 illustrates the extraction efficiency obtained with three different extraction protocols (*n*-hexane at 100 °C, t-BME at 40 °C and a mixture of petroleum ether/iso-hexane/acetone (P/I/A) at 125 °C) for fish, potato crisps, and muffins. The fat extractions gave comparable results for muffins and potato crisps. A slight difference was experienced for the smoked fish sample, for which the extrac-

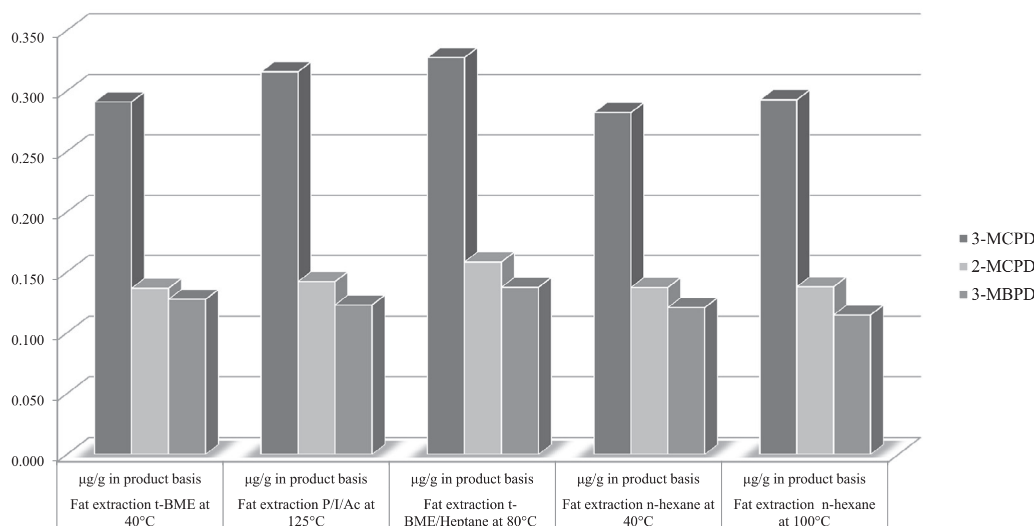

Fig. 4. Analyte content measured in extracts (expressed in $\mu g\,g^{-1}$ on product basis) obtained from a Belgian waffle sample with different extraction protocols.

tion procedure proposed by BfR (P/I/A at 125 °C) gave about 10% higher extraction yields compared to the extraction with t-BME at low temperature and with *n*-hexane at 100 °C. Even though temperature is assumed to have an influence on both the kinetics and the thermodynamics of most extraction processes [40], the overall results suggested that the calculated fat content was not influenced within the studied temperature ranges. Moreover, the use of acetone in the BfR protocol has the disadvantages of co-extracting the free forms of MCPD from samples leading to overestimation of the results, and of extracting traces of water from samples affecting the extraction efficiency of the analytes. Manirakiza et al. [40] studied the influence of the extraction method on the determination of total lipids from different food samples with a mixture of *n*-hexane and acetone (4:1). They identified hot solvent extraction as most suitable for the determination of total lipids from foods. However, this mix had the disadvantage of co-extracting water from wet samples. Therefore, it was decided to perform the extraction with 100% t-BME in two extraction cycles at a low temperature of 40 °C in order to prevent any undesirable artefact formation [17]. In addition, t-BME has proven to be sufficiently immiscible with water, hence after extraction no extra partition step was required. The average extraction efficiency of 52 extractions for the QC waffle sample, conducted over a period of 6 months was 1.092 ± 0.051 g fat/5 g sample, which corresponds to 98.4 ± 4.6% of the labelled fat content. Finally, the average extraction efficiency for all the 650 different food samples analysed in the current monitoring program and submitted to EFSA was 97 ± 10% of the labelled total fat content. These samples comprised fine bakery wares, smoked fish and meat products, fried and roasted meat, potato-based snacks and fried potato products and cereal-based snacks

Finally, extraction experiments using PLE with 100% of t-BME as extraction solvent at 40 °C were performed in triplicate for seven different food products (smoked fish, potato chips, waffles, corn flakes, smoked meat, biscuits and margarine). The powdered food samples were spiked with a mixture of native and labelled MCPDEs and GEs in toluene, and left to equilibrate for 1 h in a fume cupboard. As can be seen in Table 3, satisfactory mean recoveries were obtained. They ranged for all the studied analytes between 82 % and 114 % (Table 3). The, compared to the other matrices, lower analyte recoveries observed for the corn flakes sample are explained by the low accuracy of the determination of the fat content in this food item, which is used for transforming results from fat basis to product basis. The deficiency of accuracy in the determination of low fat contents has for the proposed analytical procedure its origin in the weighing of small amounts of fat in a large evaporation flasks. Transfer of the extract from the large into smaller evaporation vessels was avoided for the sake of sample throughput. However, the effect of potential bias in the determination of low amounts of fat was avoided by modifying the extraction procedure for test samples with expected total fat contents ≤ 5 %.

3.2. Method validation

The specificity of the method was assessed by conducting replicate analyses at various analyte content levels and with various compositions of spiked and blank edible oil samples which were subjected to the whole analysis procedure. Sufficient separation was achieved for the PBA derivatives of both 3-MCPD and 2-MCPD with the applied conditions. However, the analysis of 3-MBPD derivatives was more difficult and initial experiments showed that the signal at m/z 146 was less affected by interferences than the more abundant m/z 147. To ensure correct peak identification, at least one m/z was selected as qualifier ion based on the principle of the highest relative abundance. Attention was given to potential matrix interferences in the evaluation of the chromatograms. Chromatographic performance remained for all analytes acceptable in terms of peak resolution over the whole study period, which comprised the analysis of about 650 test samples. In addition, ion abundance ratios of target and qualifier ion peaks remained stable despite the number and composition of the samples analyzed in this study. With the exception of 3-MBPD, the selected ions for the PBA derivatives are in accordance with those reported by AOCS methods [29–31].

Nine calibration levels, as described in Section 2.7, covered the concentration range between $20\,\mu g\,kg^{-1}$ and $1850\,\mu g\,kg^{-1}$ expressed on fat basis. To avoid heteroscedasticity, the calibration range was split into two parts. The lower part, which was applicable to most of the analysed food samples, covered the range from $20\,\mu g\,kg^{-1}$ to $750\,\mu g\,kg^{-1}$, whereas a second calibration curve was set up for the range between $600\,\mu g\,kg^{-1}$ to $1850\,\mu g\,kg^{-1}$.Linearity of the instrument responses was evaluated for each section based on visual inspection of the residuals of the linear regression curves and via Mandel tests. The obtained correlation coefficients were greater than 0.999.

As the analytical method had to be fit for the generation of reliable data at low content levels emphasis was given to the assessment of the lower limits at which analytes can be detected. In the current study, limits of detection (LODs) and limits of quantifica-

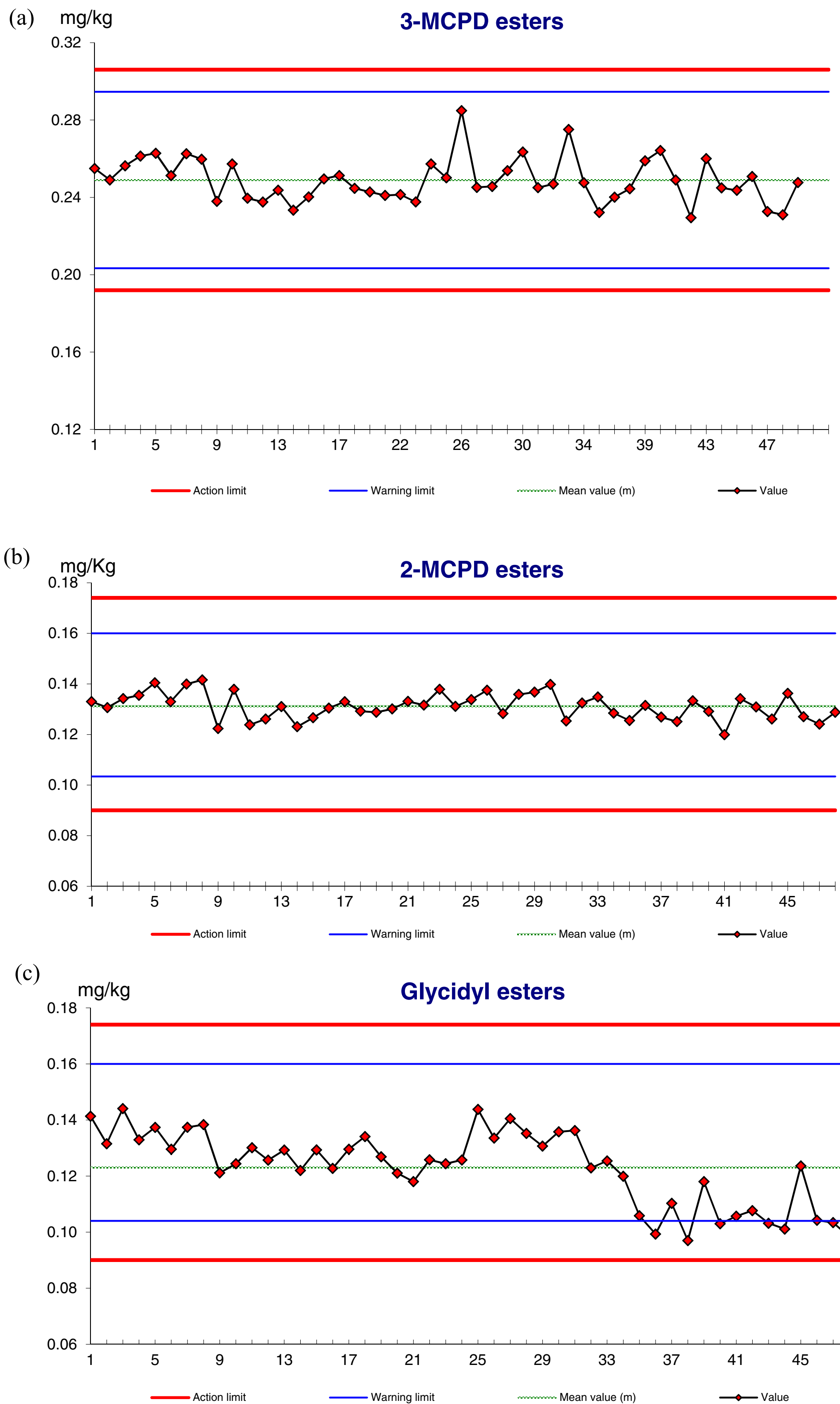

Fig. 5. Shewhart charts for the determination of (a) 3-MCPDEs (b) 2-MCPDEs and (c) GEs in the waffles QC sample over a period of six months.

tion (LOQs) were estimated via the standard deviation of ten spiked extra virgin olive oil samples corresponding to the lowest point of the calibration curve. The samples were processed applying the whole analytical procedure. Homoscedasticity was assumed for the content range between LOD and the spiking level, and the probabilities of type I and type II errors (α and ß errors) were set to 0.05. The LOD and LOQ of each analyte were calculated based on the DIN standard 32645:2008–11 [41] according to Eqs. (4) and (5), respectively. The factors 3.86 and 7.2 take into account the number of experiments and the chosen error probabilities.

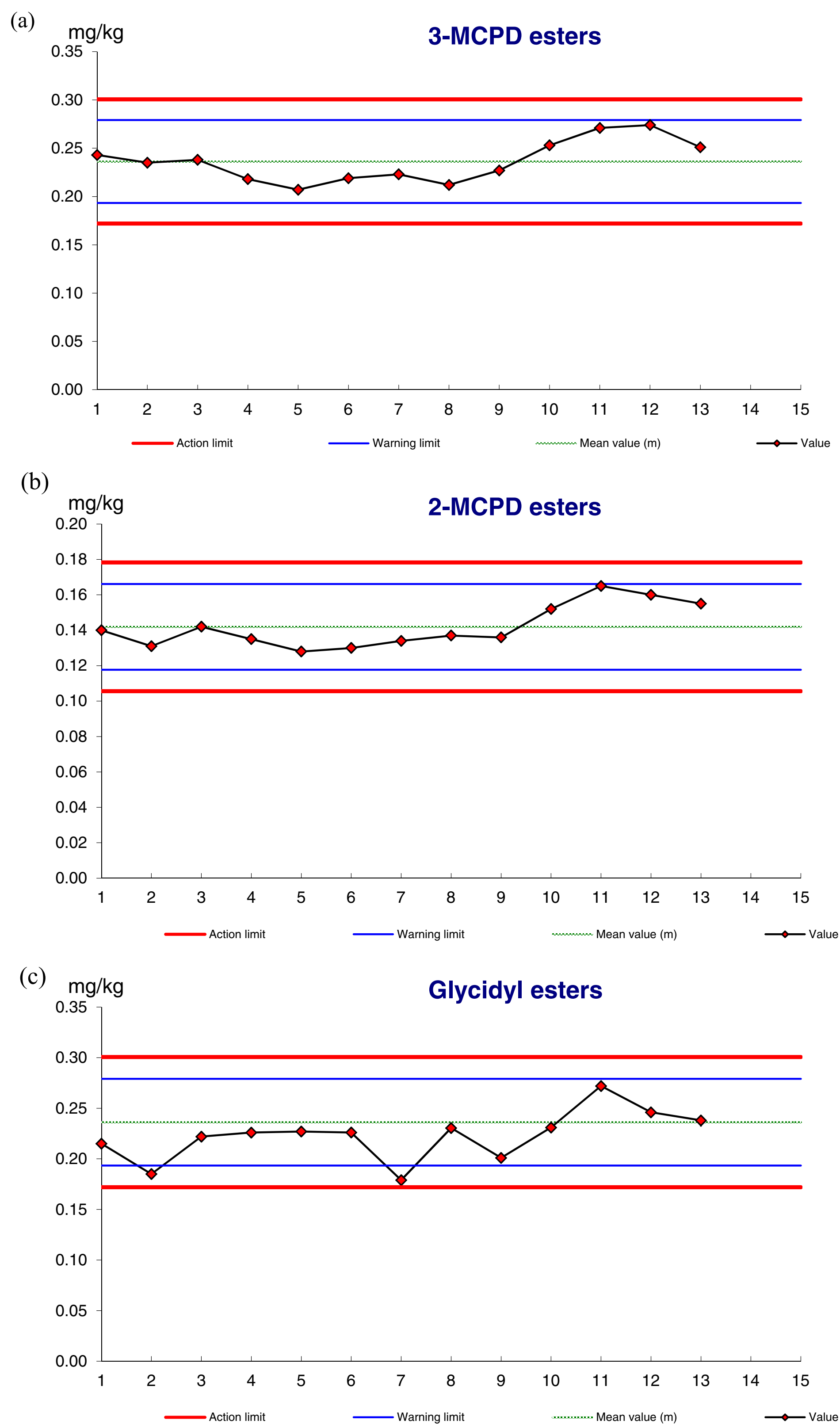

Fig. 6. Shewhart charts for the determination of (a) 3-MCPDEs, (b) 2-MCPDEs and (c) GEs in potato crisps samples (expressed in mg kg^{-1} on fat basis) over a period of six weeks.

Table 3
Mean recovery of labelled fat content ($n = 6$) and the target compounds ($n = 3$) from different food matrices. Analyte recoveries are expressed on fat basis. RSD values are given within parentheses.

	Smoked fish	Waffles	Potato chips	Corn flakes	Smoked meat	Biscuits	Margarine
Labelled fat content	19 g/100g	22 g/100g	33 g/100g	3 g/100g	15 g/100g	21 g/100g	40 g/100g
Fat recovery (%)(*six replicates*)	101 (1)	99(1)	90 (1)	41 (16)	95 (4)	98 (1)	106 (6)
Compound	Average Recoveries (%)						
3-MCPD from esters	99 (3)	98 (2)	101 (2)	83 (2)	98 (4)	98 (5)	102 (5)
2-MCPD from esters	97 (6)	100 (1)	99 (1)	82 (1)	92 (4)	99 (4)	114 (12)
3-MBPD from GEs	103 (8)	97 (2)	109 (8)	86 (2)	90 (5)	95 (9)	97 (16)

Table 4
Limits of detection (LOD) and quantification (LOQ) of MCPD esters and glycidyl esters ($\mu g\, kg^{-1}$ expressed in fat basis).

Compound	LOD $\mu g\, kg^{-1}$ fat	LOQ $\mu g\, kg^{-1}$ fat
3-MCPD from esters	7	13
2-MCPD from esters	8	15
3-MBPD from GEs	17	31

Eq. (4) was used for the estimation of LOD

$$x_{LOD} = 3.86 \times \frac{s_{y,B}}{b} \quad (4)$$

x_{LOD}: content level of LOD
$s_{y,B}$: standard deviation of the peak area of pseudo-blanks
b: slope of calibration curve
whereas Equation (5) was applied for LOQ

$$x_{LOD} = 7.2 \times \frac{s_{y,B}}{b} \quad (5)$$

x_{LOQ}: content level of LOQ
$s_{y,B}$: standard deviation of the peak area of pseudo-blanks
b: slope of calibration curve

LODs and LOQs were far below the levels set in Commission Recommendation 2014/661/EU with levels ranging from 7 to 17 $\mu g\, kg^{-1}$ and from 13 to 31 $\mu g\, kg^{-1}$ in fat (Table 4), respectively [1]. Considering the ration of fat content and sample weight, the LODs and LOQs in the current study were significantly lower than those previously obtained by other methods including the AOCS methods [29–31].

3.3. Method performance

The application of quality control measures is necessary to identify changes in performance of the analytical system at an early stage, and to demonstrate the stability of the measurements. Several parameters were considered in the setup of quality control measures.

With each batch of 10 samples, two blank samples were prepared to identify interferences, carry-over etc, which might lead to false positive results. The analyte content of the blank extra virgin olive oil was below the LOD, whereas quantifiable amounts of the analytes were not detected in any of the total 108 replicates.

In addition to that, one duplicate analysis of a randomly selected test sample was carried out from each set of 12 test samples, in order to evaluate repeatability of analysis. Moreover, data were recorded from two extra virgin olive oil samples that were spiked for evaluation of recovery with known amounts of the analytes. Results for the spiked samples as well as for QC samples were plotted on Shewhart charts. Due to the absence of long term performance data, the target standard deviation for setting warning limits and action limits were initially derived from requirements set for the determination of free 3-MCPD in soya sauce and hydrolysed vegetable protein according to the modified Horwitz equation (Commission Regulation (EC) No 1881/2006). However, the analysis results, obtained after the analysis of the first twenty QC

Table 5
Precision levels specified in Commission Regulation (EC) No 836/2011 for the determination of free 3-MCPD and precision levels applied for calculating threshold levels for QC.

Content level	Precision derived from modified Horwitz equation	Relative precision applied for QC
<120 $\mu g\, kg^{-1}$	22%	11.0%
130 $\mu g\, kg^{-1}$	21.6%	10.8%
250 $\mu g\, kg^{-1}$	19.6%	9.8%
750 $\mu g\, kg^{-1}$	16.6%	8.3%

samples, allowed lowering the thresholds to half of the original values (Table 5). The warning limits equalled the reference value, which was either represented by the spiked amount of analyte, respectively the mean of 52 and 13 replicate analyses of the waffle and potato chips QC samples, plus/minus two times the target standard deviations, whereas the action limits were formed with three times the target standard deviations. Content values were given in all charts based on the weight of the whole food.

Intermediate precision could be derived *a posteriori* from the analysis results of the commercial Belgian waffle sample which was analysed 52 times over a period of six months. From the analytical point of view, this sample was regarded as a difficult matrix since it contained palm oil, coconut oil, rapeseed oil, eggs, butter, flower, sugar, yeast, salt, water and soy lecithin. Next to naturally incurred analytes, at relevant content levels, it incorporated also substances that were expected to potentially impact reliability of results, such as lecithin that could negatively affect the extraction of fat or of the analytes. However, the established QC charts for the determination of the analytes in the waffles QC sample demonstrated that the variability of analytical results was low. The achieved intermediate precision relative standard deviations for the determination of 3-MCPDEs and 2-MCPDEs were 5% and 4% respectively, which are significantly below the 7.5% relative standard deviation used for setting the control limits (Table 5). Intermediate precision data are summarised for the different QC samples in Table 6. Consequently, the analytical procedure was considered robust for this sample. It could also be proven that the concentration levels of both 3-MCPDEs and 2-MCPDEs (Fig. 5) were stable in this matrix over the period of the project. However, the GEs content expressed as glycidol showed a negative trend from initially about 135 $\mu g\, kg^{-1}$ to 105 $\mu g\, kg^{-1}$, indicating that these analytes were not stable over a period of four months (Fig. 5). In contrast, results of the potato crisps sample, which was analysed 13 times over the first six weeks of the project indicated stability of this matrix over this short period (Fig. 6).

The trueness of the method was assessed by spiking blank extra virgin olive oil samples with MCPD esters and GEs at two concentration levels, 55 $\mu g\, kg^{-1}$ and 737 $\mu g\, kg^{-1}$, respectively. These samples were analysed 64 and 48 times over the period of the project. Results obtained for these QC samples did not indicate significant bias (Fig. S2, S3 and S4).

Table 6
Intermediate precision estimates derived from the analysis of different QC samples.

Matrix (number of replicates)	Spiked oil (low level) (*n* = 64)	Spiked oil (high level) (*n* = 48)	Waffles (*n* = 52)	Potato crisps (*n* = 13)
	% (μg kg^{-1})[a]	% (μg kg^{-1})[a]	% (μg kg^{-1})[a]	% (μg kg^{-1})[a]
3-MCPD from esters	10.6% (55)	3.7% (737)	4.6% (250)	9.1% (78)
2-MCPD from esters	5.6% (53)	4.0% (756)	4.0% (132)	8.6% (47)
3-MBPD from glycidyl esters	15.3% (55)	4.7% (720)	instable	11.0% (74)

[a] Values within brackets represent the analyte content expressed as free 3-MCPD, free 2-MCPD and glycidol. The results were expressed on whole weight basis.

3.4. Applicability of the method to real samples

The concentration levels of MCPDs and GEs were in a wide variety of tested food samples in the low microgram per kilogram range. The highest levels of both 3-MCPDEs and GEs were found in margarines followed by a potato crisps sample from sliced potatoes and fine bakery wares such as cookies, fatty cake products and puff pastries. A similar trend was observed for 2-MCPDEs with values ranging between 40 % and 60 % below those of 3-MCPD esters. Trace levels of all the target compounds were found in smoked fish, smoked meat products and fried roasted meat. In contrast, bread and bread rolls as well as cereal based products contained mostly very low/undetectable levels of MCPD esters and GEs. These findings were expected due to the low fat content of these products. The results also suggested higher concentrations in food commodities formulated with palm oil. Reporting of detailed analysis results would go beyond the scope of this paper. However, it should be stressed that all obtained analytical results were provided to EFSA for inclusion into the assessment of exposure of European citizen to MCPD esters and glycidyl esters.

4. Conclusions

This manuscript describes the performance characteristics of an analytical method for the simultaneous determination of fatty acid esters of 3-MCPD, 2-MCPD and glycidol in a wide variety of food matrices. The proposed analytical scheme proved to be efficient and robust. The design of the method allows its implementation in routine food control laboratories. Complete fat extraction and sufficient isolation of all the target compounds was achieved using t-BME as extractant at a moderate temperature of 40 °C. The obtained data confirmed compliance of method performance with performance specifications set by the European Commission. Furthermore, results for quality control samples indicated absence of significant bias and stability of performance over time.

Funding

This work was funded via a Service Level Agreement (number SLA/EFSA-JRC/DCM/2013/01) between the European Food Safety Authority (EFSA) and the Joint Research Centre (JRC).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2016.08.071.

References

[1] EC, Commission Recommendation 2014/661/EU of 10 September 2014 on the monitoring iof the presence of 2 and 3-monochloropropane-1,2-diol (2 and 3-MCPD), 2- and 3-MCPD fatty acid esters and glycidyl esters in food, OJEU L271 (2014) 93.
[2] A. Ermacora, K. Hrnčiřík, Development of an analytical method for the simultaneous analysis of MCPD esters and glycidyl esters in oil-based foodstuffs, Food Addit. Contam: Part A 31 (2014) 985–994.
[3] R. Weißhaar, R. Perz, Fatty acid esters of glycidol in refined fats and oils, Eur. J. Lipid Sci. Technol. 112 (2010) 158–165.
[4] Z. Zelinková, B. Svejkovská, J. Velíšek, M. Doležal, Fatty acid esters of 3-chloropropane-1,2-diol in edible oils, Food Addit. Contam. 23 (2006) 1290–1298.
[5] Z. Zelinková, M. Doležal, J. Velíšek, Occurrence of 3-chloropropane-1,2-diol fatty acid esters in infant and baby foods, Eur. Food Res. Technol. 228 (2008) 571–578.
[6] Z. Zelinková, O. Novotný, J. Schůrek, J. Velíšek, J. Hajšlová, M. Doležal, Occurrence of 3-MCPD fatty acid esters in human breast milk, Food Addit. Contam: Part A 25 (2008) 669–676.
[7] M. Küsters, U. Bimber, S. Reeser, R. Gallitzendörfer, M. Gerhartz, Simultaneous determination and differentiation of glycidyl esters and 3-Monochloropropane-1,2-diol (MCPD) esters in different foodstuffs by GC–MS, J. Agric. Food Chem. 59 (2011) 6263–6270.
[8] T. Buhrke, F. Frenzel, J. Kuhlmann, A. Lampen, 2-Chloro-1,3-propanediol (2-MCPD) and its fatty acid esters: cytotoxicity, metabolism, and transport by human intestinal Caco-2 cells, Arch. Toxicol. 89 (2014) 2243–2251.
[9] K. Abraham, K.E. Appel, E. Berger-Preiss, E. Apel, S. Gerling, H. Mielke, O. Creutzenberg, A. Lampen, Relative oral bioavailability of 3-MCPD from 3-MCPD fatty acid esters in rats, Arch. Toxicol. 87 (2012) 649–659.
[10] K.E. Appel, K. Abraham, E. Berger-Preiss, T. Hansen, E. Apel, S. Schuchardt, C. Vogt, N. Bakhiya, O. Creutzenberg, A. Lampen, Relative oral bioavailability of glycidol from glycidyl fatty acid esters in rats, Arch. Toxicol. 87 (2013) 1649–1659.
[11] N. Bakhiya, K. Abraham, R. Gürtler, K.E. Appel, A. Lampen, Toxicological assessment of 3-chloropropane-1,2-diol and glycidol fatty acid esters in food, Mol. Nutr. Food Res. 55 (2011) 509–521.
[12] IARC, International Agency for Research on Cancer, 2000469–486.
[13] IARC, Some Chemicals Present in Industrial and Consumer Products, Food and Drinking–Water, Vol. 101, International Agency for Research on Cancer, 2012, pp. 349–374.
[14] SCF, Opinion of the scientific committee on food on 3-monochloro-propane-1, 2-diol (3-MCPD). Updating the SCF opinion of 1994, in: Scientific Committee on Food Adopted on 30 May 2001, European Commission, Brussels, Belgium, 2001.
[15] JEFCA, 3-Chloro-1, 2-propanediol (addendum). Safety evaluation of certain food additives and contaminants. Prepared by the sixtyseventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Joint FAO/WHO Expert Committee on Food Additives WHO Food Additives Series, 58 (2007) 239–267.
[16] JEFCA, 3-Chloro-1, 2-propanediol. Safety evaluation of certain food additives and contaminants. Prepared by the fifty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Joint FAO/WHO Expert Committee on Food Additives WHO Food Additives Series, 48 (2002).
[17] C. Crews, A. Chiodini, M. Granvogl, C. Hamlet, K. Hrnčiřík, J. Kuhlmann, A. Lampen, G. Scholz, R. Weisshaar, T. Wenzl, P.R. Jasti, W. Seefelder, Analytical approaches for MCPD esters and glycidyl esters in food and biological samples: a review and future perspectives, Food Additives & Contaminants: Part A 30 (2013) 11–45.
[18] AOCS/JOCS, Glycidyl Fatty Acid Esters in Edible Oils, American Oil Chemists' Society/Japan Oil Chemists' Society, Joint AOCS/JOCS Official Method Cd, (2012) 28–10.
[19] H. Shiro, N. Kondo, N. Kibune, Y. Masukawa, Direct method for quantification of glycidol fatty acid esters in edible oils, Eur. J. Lipid Sci. Technol. 113 (2011) 356–360.
[20] M. Dubois, A. Tarres, T. Goldmann, G. Loeffelmann, A. Donaubauer, W. Seefelder, Determination of seven glycidyl esters in edible oils by gel permeation chromatography extraction and liquid chromatography coupled to mass spectrometry detection, J. Agric. Food Chem. 59 (2011) 12291–12301.
[21] T.D. Haines, K.J. Adlaf, R.M. Pierceall, I. Lee, P. Venkitasubramanian, M.W. Collison, Direct determination of MCPD fatty acid esters and glycidyl fatty acid esters in vegetable oils by LC–TOFMS, J. Am. Oil Chem. Soc. 88 (2010) 1–14.
[22] A. Ermacora, K. Hrncirik, A novel method for simultaneous monitoring of 2-MCPD, 3-MCPD and glycidyl esters in oils and fats, J. Am. Oil Chem. Soc. 90 (2012) 1–8.
[23] J. Kuhlmann, Determination of bound 2,3-epoxy-1-propanol (glycidol) and bound monochloropropanediol (MCPD) in refined oils, Eur. J. Lipid Sci. Technol. 113 (2011) 335–344.
[24] K. Koyama, K. Miyazaki, K. Abe, K. Ikuta, Y. Egawa, T. Kitta, H. Kido, T. Sano, Y. Takahashi, T. Nezu, H. Nohara, T. Miyashita, H. Yada, K. Yamazaki, Y. Watanabe, Optimization of an indirect enzymatic method for the

simultaneous analysis of 3-MCPD, 2-MCPD, and glycidyl esters in edible oils, J. Oleo. Sci. 64 (2015) 1057–1064.
[25] S.W.C. Chung, B.T.P. Chan, Simultaneous determination of 2- and 3-Monochloropropan-1,3-diol esters in foods by enzymatic hydrolysis and GC–MS detection, Chromatographia 75 (2012) 1049–1056.
[26] P.S.C.G. Hamlet, P.A. Sadd, Chloropropanols and their esters in cereal products, Czech J. Food Sci. 22 (2004) 259–262.
[27] L. Karasek, T. Wenzl, F. Ulberth, Determination of 3-MCPD esters in edible oil −methods of analysis and comparability of results, Eur. J. Lipid Sci. Technol. 113 (2011) 1433–1442.
[28] T.W.L. Karásek, F. Ulberth, Interlaboratory Comparison on the Determination of MCPD and Glycidyl Esters in Edible Oils, European Commission, JRC Scientific and policy report, 2013.
[29] A.O.C.S. (AOCS), 2- and 3-MCPD Fatty Acid Esters and Glycidol Fatty Acid Esters in Edible Oils and Fats by Acid Transesterification, AOCS Official Method Cd 29a-13, (2013).
[30] A.O.C.S. (AOCS), Determination of Bound Monochloropropanediol- (MCPD-) and Bound 2,3-epoxy-1-propanol (glycidol-) by Gas Chromatography/Mass Spectrometry (GC/MS), AOCS Official Method CD 29b-13, (2013).
[31] A.O.C.S. (AOCS), Fatty-acid-bound 3-chloropropane-1,2,diol (3-MCPD) and 2,3-epoxi-propane-1-ol (glycidol), Determination in Oils and Fats by GC/MS (Differential Measurement), AOCS Official Method Cd 29c-13, (2013).
[32] EFSA, Analysis of occurrence of 3-monochloropropane-1, 2-diol (3-MCPD) in food in Europe in the years 2009–2011 and preliminary exposure assessment, EFSA J. 11 (2013) 3381.
[33] C.S. H. Fry, A., These, A. Preiß-Weigert Collaborative study for the determination of 3- MCPD- and 2-MCPD-fatty acid esters in fat containing foods −First collaborative study part II; Method validation and proficiency test., BfR Wissenschaft, Berlin, Germany, 131 (2013) 1614–3841.
[34] H. Karl, S. Merkle, J. Kuhlmann, J. Fritsche, Development of analytical methods for the determination of free and ester bound 2-, 3-MCPD, and esterified glycidol in fishery products, Eur. J. Lipid Sci. Technol. 118 (2016) 406–417.
[35] F. Wöhrlin, H. Fry, M. Lahrssen-Wiederholt, A. Preiß-Weigert, Occurrence of fatty acid esters of 3-MCPD, 2-MCPD and glycidol in infant formula, Food Addit. Contam: Part A 32 (2015) 1810–1822.
[36] V. Divinová, M. Doležal, J. Velíšek, Free and bound 3-chloropropane-1,2-diol in coffee surrogates and malts, Czech J. Food Sci. 25 (2007) 39–47.
[37] M. Küsters, U. Bimber, A. Ossenbrüggen, S. Reeser, R. Gallitzendörfer, M. Gerhartz, Rapid and simple micromethod for the simultaneous determination of 3-MCPD and 3-MCPD esters in different foodstuffs, J. Agric. Food Chem. 58 (2010) 6570–6577.
[38] B.A. Mogol, C. Pye, W. Anderson, C. Crews, V. Gökmen, Formation of monochloropropane-1,2-diol and its esters in biscuits during baking, J. Agric. Food Chem. 62 (2014) 7297–7301.
[39] A. Dingel, R. Matissek, Esters of 3-monochloropropane-1, 2-diol and glycidol: no formation by deep frying during large-scale production of potato crisps, Eur. Food Res. Technol. 241 (2015) 719–723.
[40] P. Manirakiza, A. Covaci, P. Schepens, Comparative study on total lipid determination using soxhlet, roese-gottlieb, bligh & dyer, and modified bligh & dyer extraction methods, J. Food Compos. Anal. 14 (2001) 93–100.
[41] DIN 32645:2008–11: Chemical Analysis—Decision limit, detection limit and determination limit under repeatability conditions—Terms, methods, evaluation. Deutsches Institut für Normung: Berlin, Germany, (2008).