Determination of 3-Monochloropropanediol and Glycidol in Food Products

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Due to the increasing globalization of food markets, there are evolving new challenges for maintaing food safety. The current problem is the development of analytical methods for 3-monochloropropanediol ester and glycidol ester, which are food contaminants of concern for the scientific community. The levels of 3-monochloropropanediol ester and glycidol ester in certain food products are controlled by the European legislation. However, the maximum allowed concentrations and uptake limits for various food products are permanently revised. Therefore, we aimed to determine 3-monochloropropanediol ester and glycidol ester in various food products, which may contain vegetable oils. We analyzed food samples obtained from local food shops, predominantly low-priced products, which are more likely to contain vegetable oils, and adulterated milk fat. The levels of 3-monochloropropanediol ester and glycidol ester were determined indirectly by analyzing free 3-monochloropropanediol and glycidol ester obtained by hydrolysis and derivatized with phenylboronic acid. Samples were analyzed by GC-MS/MS on a triple-quad mass spectrometer.

Keywords: 3-Monochloropropanediol, Glycidol, Contaminants, Food products, Gas chromatography, Tandem mass spectrometry.

INTRODUCTION

3-Monochloropropanediol esters (3-MCPDEs) and glycidyl esters (GEs) are the technogenic contaminants formed from naturally occurring acylglycerols and diacylglycerols during high-temperature processing (refining or deodorizing) of vegetable oils or during food processing [1-3]. According to the European Food Safety Authority (EFSA), consuming of food contaminated with 3-MCPDEs and GEs are harmful for humans, especially at a young age. Glycidyl esters (GEs) were proved genotoxic, carcinogenic and mutagenic [4]. Both EFSA and World Health Organization (WHO) confirmed that ingested GEs are harmful for human health.

Any refined vegetable oils may contain 3-MCPDEs, although the highest concentrations were observed in refined palm oil [5]. Analytical methods for GEs and 3-MCPDEs can be classified either direct or indirect. Direct methods refer to the direct detection and quantification of intact GEs or 3-MCPDEs without any chemical modification in sample preparation. Generally, GEs and 3-MCPDEs are separated from acylglycerols by gel permeation or double solid-phase extractions, or the sample could be diluted to reduce the interference from acylglycerols in oil matrix. By using direct methods, the composition and levels of each species of GEs and 3-MCPDEs with different fatty acyl groups can be obtained without interference from potential side reactions. However, expensive reference standards are required for analysis of every possible species of GEs and 3-MCPDEs. Also, coelution of 2-MCPDEs and 3-MCPDEs limits the use of direct methods for routine analysis. Indirect methods are based on the conversion of GEs and 3-MCPDEs into their free forms, glycidol and 3-MCPD, by chemical (alkaline or acidic) or enzymatic hydrolysis. The released glycidol and 3-MCPD generally undergoes an additional halogenation step to convert glycidol into 3-MCPD or 3-bromo-1,2-propanediol (3-MBPD) for derivatization by phenylboronic acid (PBA). Quantification is achieved by determining PBA derivatives of 3-MCPD (from 3-MCPDEs) or 3-MBPD (from GEs) by gas chromatography-mass spectrometry (GC-MS). The content of GEs and 3-MCPDEs determined by indirect methods is usually expressed as the content or equivalent of glycidol and 3-MCPD (free form of GEs and 3-MCPDEs), respectively. Indirect methods require much fewer reference standards than direct methods.

Therefore, indirect methods are preferred for routine analysis [6].

Currently, two possible mechanisms of MCPDE formation are available in the literature [7], both of which involve acyl-oxonium ions as reactive intermediates. The first step is a reaction between HCl, which is formed by the thermal decomposition of organochlorine compounds present in the oil, and a carbonyl group of a triacylglycerol to form an acyloxonium ion. Next, this acyloxonium intermediate may rearrange into a cyclic form with the elimination of a fatty acid molecule (cyclic acyloxonium pathway). A nucleophilic substitution with Cl⁻ on either S_N1 or S_N3 carbons of this cyclic intermediate would result in the formation of MCPD diesters. Alternatively, the diesters can be formed by direct nucleophilic attack of Cl⁻ on the open-chain acyloxonium intermediate with the release of a fatty acid molecule.

Similarly, to MCPDEs, GEs may form in oils during de-odorizing. It was demonstrated [8,9] that GEs are produced from mono- and diacylglycerols at temperatures exceeding 200 ºC. Animal studies show that the 3-MCPDEs are hydrolyzed in the gastrointestinal tract to free 3-MCPD, which is toxic. Similar to 3-MCPDEs, GEs after consumption are hydrolyzed to glycidol in the gastrointestinal tract. Glycidol was shown to be genotoxic and carcinogenic during animal studies [10].

Since the highest concentrations of GEs and 3-MCPDEs occur in palm oil [5], this oil may be the main source of food contamination with GEs and 3-MCPDEs. The addition of palm oil may be illegal, *e.g.* in adulterated dairy products in which milk fat is either totally or partially replaced with low-grade vegetable oils. Therefore, an analytical method is developed to determine GEs and 3-MCPDEs in food, with emphasis on dairy products.

EXPERIMENTAL

Chemicals: Standards, 1,2-bis-palmitoyl-3-chloropropanediol-D5, 1,2-bis-palmitoyl-3-chloropropanediol, 3-chloro-1,2-propanediol and glycidyl stearate were procured from Toronto Research Chemicals (North York, Canada). Solvents used in this study including toluene, *tertiary*-butyl methyl ether (*t*-BME), methanol, *n*-hexane, ethyl acetate, diethyl ether and *iso*-octane were purchased from Merck (Darmstadt, Germany). Sodium hydroxide, sodium chloride, sodium bromide sodium sulfate and phenylboronic acid were procured from Sigma-Aldrich (St. Louis, MO, USA).

Sample preparation: Food samples obtained from local shops were analyzed. Low-priced products were preferred as they are more likely to contain vegetable oils and adulterated milk fat.

3-Monochloropropanediol ester (3-MCPDEs) were determined indirectly by measuring 3-monochloropropanediol (3-MCPD) obtained by hydrolysis and derivatized with phenyl boronic acid.

The sample preparation consisted of several steps. First, the samples were mixed with the deuterium-labelled internal standard (D5-3-MCPD-1,2-bispalmitoyl ester) and extracted with methyl *tert*-butyl ether. Next, glycidol and 3-MCPD were determined in sample A and sample B, respectively. The samples

were hydrolyzed with methanolic NaOH, then either acidified NaCl solution (sample A) or NaBr solution (sample B) were added, and the reaction mixture extracted twice with *n*-hexane, then three times with diethyl ether/ethyl acetate and dried with anhydrous sodium sulfate. For derivatization, a saturated solution of phenylboronic acid in diethyl ether was added, evaporated to dryness and re-dissolved in isooctane.

Samples were analyzed by GC-MS/MS on a TSQ 8000 Evo triple-quad mass spectrometer (Thermo Fisher Scientific, USA) equipped with a HP-50 capillary column (30 m × 0.25 mm, 0.25 µm liquid phase) from Agilent Technologies, USA. Chromatographic separation conditions were performed according to ISO 18363-1:2015 [11]. Mass spectrometric detection was done by MRM method in EI mode using the characteristic ions listed in Table-1.

TABLE-1
CHARACTERISTIC IONS USED IN MASS SPECTROMETRIC DETECTION

Analyte	Parent ion (*m/z*)	Daughter ion (*m/z*)
3-MCPD	147	91
	196	91
3-MCPD-d₅	150	93
	201	93

The mass spectrometric data were analyzed using Xcalibur 4.0.27.10 software (Thermo Fisher Scientific, USA).

RESULTS AND DISCUSSION

The data obtained during the in-house validation of the procedure are shown in Tables 2 and 3.

TABLE-2
LINEARITY, SELECTIVITY, LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

Analyte	Linearity	Selectivity	LOD (mg/kg)	LOQ (mg/kg)
3-MCPD ester	0.1-5 mg/kg $y = 3.5573x + 0.5654$ $R^2 = 0.9997$	No matrix effects	0.005	0.01
Glycidyl ester	0.1-5 mg/kg $y = 8.4496x + 0.8405$ $R^2 = 0.9983$	No matrix effects	0.005	0.01

TABLE-3
RECOVERY AND PRECISION

Analyte	Spike level (mg/kg)	R (%)	CV$_r$ (%)	CV$_R$ (%)
3-MCPD ester	0.1	97	11.9	18.3
	1.0	95	10.7	15.1
	2.0	89	7.2	13.3
	5.0	82	6.8	11.0
Glycidyl ester	0.1	75	12.5	15.7
	1.0	69	10.5	12.3
	2.0	82	8.7	8.9
	5.0	69	6.8	6.2

R = recovery (12 replicates for each spike level); CV$_r$ = coefficient of variation under repeatability conditions (same day, six replicates for each spike level); CV$_R$ = coefficient of variation under within-laboratory reproducibility conditions (same laboratory, different days, 12 replicates for each spike level).

TABLE-4
LEVELS OF 3-MCPD AND GLYCIDYL ESTERS IN FOOD (N = 76)

Sample type	N	Glycidyl esters (mg/kg)			3-MCPD esters		
		N > LOQ	LB–UB	Mean	N > LOQ	LB–UB	Mean
French fries	7	5	< 0.010 – 0.20	0.051	7	< 0.010 – 0.32	0.11
Potato chips	9	4	< 0.010 – 0.13	0.063	8	< 0.010 – 0.41	0.24
Cottage cheese cakes	2	2	0.47 – 2.2	1.3	2	0.28 – 1.7	0.99
Waffles	13	11	< 0.010 – 0.72	0.38	10	< 0.010 – 0.55	0.29
Cream cake	11	7	< 0.010 – 0.33	0.18	9	< 0.010 – 0.67	0.32
Croissants	3	0	nd – < 0.010	–	1	nd – 0.11	0.11
Sprats	1	0	< 0.010	–	1	0.060	0.060
Pancakes	2	0	nd	–	2	0.080 – 0.10	0.090
Ice cream	9	7	< 0.010 – 2.8	1.1	7	< 0.010 – 0.75	0.48
Cheese	10	5	nd – 0.82	0.33	9	< 0.010 – 1.3	0.79
Biscuits	8	3	nd – 0.14	0.085	5	< 0.010 – 0.50	0.23
Vegetable dry milk	1	1	0.52	–	1	2.3	–

The method showed good linearity in the investigated range of concentrations and no matrix effects. The LOD and LOQ can be considered satisfactory for the determination of low concentrations of the compounds. Recovery ranged from 69 to 97%.

This method was applied to 76 different food samples and the results are shown in Table-4. The levels of 3-MCPD ester varied from not detected to 2.3 mg/kg. The levels of glycidyl ester varied from not detected to 2.8 mg/kg.

Conclusion

Present results indicate that analyzed dairy products were contaminated with 3-monochloropropanediol esters (3-MCP-DEs) and glycidyl esters (GEs). One plausible explanation is that low-grade vegetable oils were used for milk fat adulteration. Although the formation of 3-MCPDEs and GEs during high-temperature processing of oils and fats was reported in the literature, the results also suggested that these contaminants are not formed in particularly high concentrations at temperatures used for cooking and baking.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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