Levels of PCDDs/PCDFs in several cheese and butter samples collected from Cluj-Napoca market, Romania

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Abstract. Consumption of foods, especially those high in fat represents the main route of exposure of the human body to polychlorodibenzo-p-dioxins (PCDDs) and polychloro-dibenzofurans (PCDFs). Since June 2014, the European legislation recognized gas chromatography coupled with triple quadrupole mass spectrometry (GC-MS/MS) as a confirmatory tool for the presence of dioxins and dioxin-like compounds and their contents against the maximum levels. In this study, the contents of PCDDs and PCDFs in dairy foods (3 cheese and 3 butter samples) collected from Cluj-Napoca market (Romania) were determined using GC-MS/MS and isotope dilution technique. For the extraction of these compounds from the food fatty matrix, the accelerated solvent extraction was used, then the extract was purified in order to isolate the fraction PCDDs/PCDFs with an automated system. The results were expressed as total toxicity equivalent (TEQ), calculated on the basis of toxicity equivalency factors (TEF), according to the World Health Organization. For butter and cheese, the mean values were 0.060 and 0.134 pg WHO-TEQ/g fat respectively, lower than the maximum levels for PCDDs/PCDFs imposed by EU legislation in milk products.

Key Words: PCDD/Fs, GC-MS/MS, accelerated solvent extraction (ASE), cheese, butter.

Introduction. Dioxins represent a term used for a selected group of 210 different chlorinated compounds consisting of the polychlorinated dibenzo-p-dioxins (PCDD, 75 congeners) and the closely related polychlorinated dibenzofurans (PCDF, 135 congeners). Dioxins are mainly unwanted by-products of a wide range of industrial processes including smelting, chlorine bleaching of paper pulp, manufacturing of some herbicides and pesticides, and are formed also in almost every combustion process (Hoogenboom et al 2015). In terms of dioxin released into the environment, uncontrolled waste incinerators are often the major source due to incomplete burning (Zhang et al 2016). Long term storage and improper disposal of PCB-based waste industrial oils, many with high levels of PCDFs, may result also in dioxin release into the environment and consequently the contamination of human and animal food supplies (Gupta 2015).

Although formation of dioxins is local, environmental distribution is global. Dioxins are found throughout the world in the environment. The highest levels of these compounds are found in some soils (Tue et al 2016), sediments (Burniston et al 2015) and animal originated foodstuffs (Hoang et al 2014; Sobek 2014). Very low levels are found in plants, water and air (Li et al 2009; Coutinho et al 2015).

Especially the 17 congeners with chlorine atoms at the 2, 3, 7 and 8 positions appear to be of most importance since they are relatively resistant to metabolic degradation and they accumulate in the body. All compounds of these chemical classes are highly lipophilic and persistent due to chemical inertness (Schuhmacher et al 2013). They tend to bioaccumulate through the trophic chain and pose a threat to human health. Thus, the consumption of animal products with high fat content, such as meat, eggs and dairy products, can increase human exposure to dioxins. Recent studies showed that around 95% of human exposure to dioxin occurs through consumption of food of animal

origin, such as meat, dairy products and fish (Ghimpeteanu et al 2014). Concerns regarding the exposure to these contaminants and their toxicity capacity exist because these compounds are considered to have the potential to cause endocrine system disruption, skin lesions, immune system damage, reproductive disorders and cancer even at background levels of exposure (Wittsiepe et al 2015). Consequently, accurate detection and quantification of PCDDs/PCDFs in the environment, particularly in food and animal feed become important.

Until recently, the high-resolution gas chromatography/mass spectrometry (HRGC/HRMS) was the only option and the standard method for detecting, confirming and quantification of dioxin and dioxin-like compounds in contaminated samples (Petronijevic et al 2015). Recent technological advances in gas chromatography/triple-quadrupole mass spectrometry (GC-QQQMS/MS) technology have led, starting with 23rd of June 2014, to recognize it as a reliable tool that can be used to control the maximum levels (MLs) for PCDDs/PCDFs in food and feed as a full confirmatory method (Application Note 10380; L'Homme et al 2015).

Because human exposure to dioxins occurs mostly from ingesting fatty foods, the aim of this study was to evaluate PCDDs/PCDFs presence in commercially available common used dairy foodstuffs using GC-MS/MS.

Material and Method. A number of 6 dairy food samples, including 3 types of butter (two with 65% and one with 82% fat content), 2 types of melted cheese (23%, 24% fat content, respectively) and 1 type of pressed cheese (25% fat) were collected from local market in Cluj-Napoca, Romania. The food items were sampled in their genuine package, were brought to the laboratory and subsequently analyzed.

Solvents (hexane, toluene, dichloromethane) SupraSolv were for gas chromatography (Merck, Darmstadt, Germany). Ultrapure water (18.2 MΩ cm) was prepared by a Direct Q UV 3 Millipore system (Bedford, MA, USA). The disposable columns (multi-layer acidic silica gel column, Florisil column, large and small carbon columns) for the automated clean-up were obtained from LCTech (Germany).

All congeners of PCDDs/PCDFs were quantitated against their own 13C-labeled internal standards. The calibration curves - 5 levels - for PCDDs/PCDFs were prepared using CIL-EDF-4947 Calibration Solutions [CS1-CS5]. Extraction and Syringe recoveries were measured with Extraction Standard Solution (CIL-EDF-4139) and Syringe Standard Solution (CIL-ED-4140) (CIL). All the standard solutions were acquisitioned from LGC Standards (Germany) and were distributed by Cambridge Isotopic Laboratories (Cerilliant, CIL, Round Rock, TX, USA).

ASE Prep DE (diatomaceous earth) (Thermo Scientific) was used in the extraction cell, during the extraction stage. Pure grade helium gas, 99.9999% was purchased from Linde Gas (Timisoara, Romania).

Samples were weighed using an AS 60/220.R2 analytical balance (Radwag, Poland). The extraction of PCDDs/PCDFs from dairy samples was performed using an Accelerated Solvent Extractor (ASE 350, Dionex USA) equipped with 22 mL stainless steel extraction cells, cellulose extraction filters (Thermo Scientific, USA) and 250 mL clear collection bottles and supplied with 99.999% nitrogen (Linde Gas, Romania). The extracts were further purified using an automated sample clean-up system (DexTech, LCTech, Germany) on 4 columns. Rotary evaporator (Laborota 4010, Heidolph, Germany) coupled with a vacuum pump (Ilmvac, Germany) was used for extract concentration. For PCDDs/PCDFs analysis, a gas chromatograph coupled with triple quadrupole mass spectrometer (Trace 1300 GC/TSQ 8000, Thermo Scientific, USA) equipped with a Programmable Temperature Vaporizer (PTV)-based injector, TriPlus RSH autosampler and a Thermo Scientific TraceGOLD TG-5SilMS (60 m × 0.25 mm I.D. × 0.25 µm) capillary column was used.

An amount of 5-6 g of food samples, weighted with 0.1 mg accuracy was homogenized and mixed with diatomaceous earth and placed in the ASE extraction cell. Before extraction, samples were spiked with internal standard containing native and 13C12-labeled PCDDs and PCDFs. The fat extraction was carried out using an ASE. The extraction solvents were toluene:hexane (1:1) at a temperature of 120°C and pressure

of 120 bar with two cycle of extraction. The resulting extract was passed through automated sample clean-up equipment using four columns. The extraction volume was concentrated to 2 mL on the rotary evaporator. Finally, samples were dried under gentle N_2 flow and reconstituted with 10 µL of nonane and transferred to vials adding 15 µL of internal standard.

The concentrated sample extract was injected into the GC-MS/MS system, with the following oven temperature program: 120°C with 2 min hold time, followed by an increases with 40°C min^{-1} up to 220°C and maintained at this temperature for 15 minutes, increased again with 2.3°C min^{-1} until 250°C and hold at this temperature for 13.5 minutes, followed by a new increase with 20°C min^{-1} up to 310°C and hold at this final temperature for 10 minutes. The transfer line temperature and ion source temperature were set at 270 and 250°C, respectively.

Data processing was performed using TargetQuan software which make quantitation based upon relative response factors, incorporating toxic equivalence factors (TEFs) to automatically calculate toxic equivalence quotients (TEQs), and finally determines total TEQ.

The concentrations are expressed as World Health Organisation (WHO) toxic equivalents (TEQs) using WHO-2005-TEF.

Results and Discussion. The concentrations of sum of PCDDs/PCDFs in the dairy food are shown in Table 1. They were expressed as World Health Organisation (WHO) toxic equivalent using the WHO-toxic equivalency factors (WHO-TEFs). WHO-TEFs for human risk assessment are based on the conclusions of the World Health Organisation (WHO) - International Programme on Chemical Safety (IPCS) expert meeting which was held in Geneva in June 2005 (Van der Berg et al 2006).

The obtained concentrations for the sum of PCDDs/PCDFs in the investigated samples were far below the EU's maximum level of 2.5 pg/g fat in dairy products (EC 1259/2011).

Table 1

Contents of sum of dioxins and furans (PCDDs/PCDFs) (pg WHO-TEQ/g fat, using TEF, 2005)

Crt. no	*Sample*	*PCDD/Fs, pg WHO-TEQ/g fat*
1	Butter, 65% fat	0.027
2	Butter, 65% fat	0.011
3	Butter, 82% fat	0.142
4	Melted cheese, 23% fat	0.054
5	Melted cheese, 24% fat	0.243
6	Pressed cheese, 25% fat	0.106

In butter samples, the dominant congeners were 12378-PeCDD, 1234678-HpCDD, 123478-HxCDF, 123678-HxCDF and 234678-HxCDF. In cheese samples, the 12378-PeCDF and 1234678-HpCDD were the most abundant congeners.

The obtained values were lower than those reported by Pizarro-Aránguiz et al (2015) for dairy products from Chile during the 2011-2013 survey. Also, PCDDs/PCDFs concentrations in butter samples recorded in our study were lower than those obtained by Malisch & Dilara (2007) in butter samples from European Union countries.

The mean concentration of PCDDs/PCDFs in cheese samples (0.134 pg WHO-TEQ/g fat) in our study was higher than that obtained in cheese (0.081 pg WHO-TEQ/g fat) from Taiwan markets, but the mean concentration of PCDDs/PCDFs in butter samples (0.060 pg WHO-TEQ/g fat) was lower than concentration in butter (0.338 pg WHO-TEQ/g fat) collected in Taiwan markets from 2004 to 2012 (Lee et al 2016).

Conclusions. This study regarding the PCDDs/PCDFs content in 6 dairy products (3 butter and 3 cheese items) collected from the Cluj-Napoca market has demonstrated that these products are safe for consumption. The concentrations of PCDDs/PCDFs were low compared to maximum levels laid down in European regulations.

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