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First study on trichothecene and zearalenone exposure of the Romanian population through wheat-based products consumption

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ABSTRACT

In this study, a dietary exposure assessment of mycotoxins was conducted for the Romanian population using the contamination data of a various categories of wheat-based products for direct human consumption. Wheat-based foods (n = 181) commercialized in Romania, including flour, bread, biscuits, breakfast cereals and pasta, were evaluated by GC-QqQ-MS/MS for the occurrence of deoxynivalenol (DON), 3-acetyldeoxynivalenol (3AcDON), 15-acetyldeoxynivalenol (15AcDON), fusarenon-X, nivalenol, HT-2 and T-2 toxins, diacetoxyscirpenol, neosolaniol and zearalenone (ZEA). DON and 15AcDON were detected in 63 and 5% of all the analyzed samples, whereas 13-AcDON, HT-2, T-2, NIV and ZEA were not detected. Exposure of Romanian adult population was assessed, the EDIs for the sum of DON + 3AcDON + 15AcDON were 669 ng kg^{-1} bw day^{-1} at low-bound estimation, and 690 ng kg^{-1} bw day^{-1} at upper-bound estimation, being lower than the TDI set (1000 ng kg^{-1} bw day^{-1}).

1. Introduction

The accumulation of mycotoxins in foods represents a major threat for human health as they are responsible for various toxicities, and also it has a high economic significance (Adeyeye, 2016). The presence of mycotoxins in the food chain is considered a human public health issue, affecting infants, children and adults (Lee and Ryu, 2015; Ortiz et al., 2018). Acute exposure to high levels of mycotoxins is not very common, but the adverse effects in chronic exposure continue to attract worldwide attention because of their impact on human health (Escrivá et al., 2015; Marin et al., 2013).

Even if mycotoxins are specific contaminants for cereals, they can be ingested by humans consuming various foodstuffs. From cereals, mycotoxins can be transferred directly to food products of vegetal origin, or they can be found in food products of animal origin (Stanciu et al., 2015). Simultaneous presence of more than one mycotoxin in food products may represent a real risk due to potential additive, antagonistic, or synergistic toxic effects, sometimes thoughtless. Hence, a new topic represented by developing validated multi-mycotoxin analytical methods became important in the last years, with the goal to obtain an accurate assessment of human exposure to mycotoxins (Berthiller et al., 2017; De Santis et al., 2017; Mousavi Khaneghah et al., 2018).

Risks associated with mycotoxins depend on both hazard and exposure. It is indispensable from a public health perspective to develop acceptable methods to assess human risk associated to the presence of mycotoxins in food.

To assess the exposure to a mycotoxin, estimated daily intake (EDI) is calculated. It is expressed in ng kg^{-1} body weight day^{-1} (ng kg bw^{-1} day^{-1}) and for its calculus is necessary to know the mycotoxin level in food or in a group of food commodities, and the dietary habits of the population in the region studied. The current exposure assessment designs are largely deterministic and uncertainty and/or variability issues are accounted for by means of cautionary measures which are implicitly

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embedded in calculation designs. More recently, probabilistic methods as Monte Carlo simulations have been developed to quantify the sources of uncertainty and variability of human exposure (Cano-Sancho et al., 2010; Kettler et al., 2015). Mycotoxin exposure can be evaluated also by specific and suitable biomarkers of exposure for the human body (biomonitoring) (Lee and Ryu, 2015; Rodríguez-Carrasco et al., 2014a; Escrivá et al., 2017).

Based on the toxicological experiments, and taking into account the total human diet and the possibilities for mycotoxin intake, legislation concerning tolerable daily intake (TDI) of mycotoxins has been published. The Joint FAO/WHO Expert Committee in Food Additives (JECFA), the Scientific Committee on Food (SCF), and the European Food Safety Authority (EFSA) have proposed TDI values for deoxynivalenol (DON) (1000 ng kg bw^{-1} day^{-1}) (SCF, 2002) and zearalenone (ZEA) (200 ng kg bw^{-1} day^{-1}) (SCF, 2000), and provisional maximum TDIs (PMTDI) for nivalenol (NIV) (700 ng kg bw^{-1} day^{-1}) (SCF, 2002), the sum of DON, 3 and 15 acetyldeoxynivalenol (1000 ng kg bw^{-1} day^{-1}) (JECFA, 2010), and the sum of T-2 and HT-2 toxins (100 ng kg bw^{-1} day^{-1}) (EFSA, 2011).

Monitoring exposure to various mycotoxins has become a key part of ensuring food safety. Dietary exposure is defined as the amount of a certain substance that is consumed and is usually estimated by combining food consumption data with data on the concentration of chemicals in food (IPCS, 2009). The surveillance and exposure studies are indispensable for human health concern and these investigations gain higher importance for vulnerable groups in the population, such as babies and young children (Ortiz et al., 2018).

Taking into account the Romanian background in the field of mycotoxin research in products for direct human consumption, the aims of this study were: (i) to validate a selective multi-mycotoxin GC-QqQ-MS/MS method for the analysis of nine trichothecenes and ZEA in flour, pasta, breakfast wheat-based cereals, bread and biscuits; (ii) to survey the levels of these mycotoxins in wheat-based products commercialized in Romania; (iii) to estimate the daily intake of mycotoxins through wheat-based product consumption for the Romanian population. This is the first survey concerning the exposure assessment of both legislated and non-legislated mycotoxins through wheat-based product consumption by the Romanian population.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile and hexane were supplied by Merck KGaA (Darmstadt, Germany), whereas methanol MS/MS grade (≥99.9% purity) was supplied by VWR International Eurolab (Barcelona, Spain). Deionized water (< 10 MΩ cm^{-1} resistivity) was manufactured in the laboratory using a Milli-Q SP® Reagent Water System (Millipore, Bedford, MA, USA). The derivatisation reagent BSA (N,O-bis(trimethylsilyl) acetamide) + TMCS (trimethylchlorosilane) + TMSI (N− trimethylsilylimidazole) (3:2:3) was purchased from Supelco (Bellefonte, PA, USA). Sodium dihydrogen phosphate and disodium hydrogen phosphate, used to prepare phosphate buffer, were acquired from PanReac AppliChem (Castellar del Vallés, Spain).

Whatman no. 4 filter papers (Maidstone, UK) were used to filter the extract samples. Polypropylene syringes (2 mL) and nylon filters (13 mm diameter, 0.22 μm pore size) were purchased from Análisis Vínicos S.L. (Tomelloso, Spain).

The certified standards of DON, NIV, 3 acetyldeoxinivalenol (3AcDON), 15 acetyldeoxinivalenol (15AcDON), fusarenon-X (FUS-X), HT-2 toxin (HT-2), T-2 toxin (T-2), neosolaniol (NEO), diacetoxyscirpenol (DAS) and ZEA were purchased from Sigma Aldrich (Madrid, Spain). The individual stock solutions of all mycotoxins were prepared in acetonitrile at 1000 μg mL^{-1}. A working mixed standard solution in acetonitrile at concentration of 100 μg mL^{-1} was prepared. This solution was used to prepare the calibration curves, matrix matched calibration curves, and for the method validation. For the quantification of samples, each corresponding matrix-matched calibration curves were used (wheat, pasta, breakfast cereal, wheat flour and integral biscuit). The solutions were kept in glass-stoppered bottles, protected from light at −20 °C.

2.2. Sampling

A total of 181 samples of wheat-based products were purchased from different markets located in Cluj-Napoca (Romania) during April to October 2016: white wheat flour (n = 41); pasta with minimum of 73% wheat (n = 40); breakfast cereals containing between 54% and 90% wheat (n = 7); integral biscuits containing between 49% and 95% wheat (n = 23); wheat flour-based bread (n = 70). Two to six packages for each sample were purchased, obtaining an aggregate sample of at least 1 kg total weight. All samples were milled to a fine powder using a laboratory mill. After homogenization, 500 g samples were packed in plastic bags and kept at −20 °C in a dark and dry place until analysis. Three replicates for each sample were weighed for analysis.

2.3. Sample preparation

2.3.1. Extraction

Extraction was performed according to the procedure described by Stanciu et al. (2017), using solid liquid extraction. Mycotoxin-free powdered samples were used as blank material for validation study. All experiments were performed in triplicate. After residue reconstitution and filtration, a volume of 200 μL of filtrate was placed in a chromatographic vial and was evaporated to dryness at 35 °C under a gentle stream of nitrogen using a multi-sample Turbovap LV Evaporator (Zymark, Hoptkinton, MA, USA).

2.3.2. Derivatisation

Over the dry extract 50 μL of derivatisation reagent were added and the sample was left for 30 min at laboratory temperature. The derivatised sample was diluted to 200 μL with hexane and mixed thoroughly on a vortex for 30 s. Then the hexane was washed with 1 mL of phosphate buffer (60 mM, pH 7) and mixed until the upper layer was clear. Finally, the upper layer was transferred to an autosampler vial for chromatographic analysis.

2.4. GC-QqQ-MS/MS

The analysis was carried out using an Agilent 7890A GC system with electron ionization source (70eV) coupled to an Agilent 7000A triple quadrupole mass spectrometer and an Agilent 7693 autosampler (Agilent Technologies, Palo Alto, CA, USA). Quantitative data were acquired at selection reaction monitoring mode. The transfer line and source temperatures were 280 and 230 °C, respectively. The collision gas for MS/MS experiments was nitrogen (1.5 mL min^{-1}), and helium was used as the quenching gas (2.25 mL min^{-1}), both at 99.999% purity supplied by Carburos Metálicos S.L. (Barcelona, Spain). Data were acquired and processed using the Agilent Masshunter version B.04.00 software.

Analytes were separated on a HP-5MS 30 m × 0.25 mm × 0.25 μm capillary column. A total of 1 μL of extract was injected in splitless mode in programmable temperature vaporization inlet (150 °C for 0.1 min then 600 °C min^{-1} to 250 °C for 5 min) employing helium as the carrier gas at a fixed pressure of 20.3 psi. The oven temperature programme was initially 80 °C, and the temperature was increased to 245 °C at 60 °C min^{-1}. After a 3 min hold time, the temperature was increased to 260 °C at 3 °C min^{-1} and finally to 270 °C at 10 °C min^{-1} and then held for 10 min.