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Presence of mycotoxins in Tunisian infant foods samples and subsequent risk assessment

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ABSTRACT

A study on a set of cereal and cereal-based products (n = 117) intended for infant consumption was performed to determine the natural presence of twenty different mycotoxins by both liquid chromatography and gas chromatography coupled to tandem mass spectrometry (MS/MS). Analytical data showed that 67% of analyzed samples were contaminated by at least one mycotoxin at variable levels. Deoxynivalenol presented the highest incidence (38%) and reached a maximum level of 240 ng/g in a bsissa sample (oat product), higher than the maximum level set for DON in cereal products (200 ng/g). While, enniatin B was found in 25 samples with a maximum level of 316 ng/g found also in a bsissa sample. Furthermore, 32% of positive samples showed co-occurrence of at least two mycotoxins, and a combination of up to six different mycotoxins was found within the positive samples. It was also observed that only the intake of bsissa and sorghum samples may pose a real OTA and HT-2 toxin health risk to the high consumers. Overall, no toxicological concern was associated to mycotoxins exposure for infant population; but a special attention should be paid to samples with co-occurring mycotoxins, where the estimated daily intake increase considerably for high consumers.

1. Introduction

Cereals and derived products represent worldwide the main babies' diets, providing them energy and nutrients, sustaining their expected growth and development. In order to offer safety cereal foodstuffs, it is necessary to ensure the quality of the raw cereals, by analytical control and implementing hazard analysis and critical control point (HACCP). Cereals may be subject to contamination by microorganisms including fungi that could accompany the matrix throughout the production chain. Several fungi (mainly *Aspergillus*, *Penicillium* and *Fusarium*) are able to produce, under favourable environmental conditions, secondary metabolites with low molecular weight termed mycotoxins (Oueslati, Romero-Gonzalez, Lasram, Frenich, & Vidal, 2012; Petzinger & Weindenbach, 2002). The mycotoxins exposure is a worldwide concern due to the globalization of food trade (Anfossi, Giovannoli, & Baggiani, 2016). Some of these toxins are hepatotoxic, genotoxic, immunosuppressive, nephrotoxic, teratogenic, and/or carcinogenic effects (Metzler, Pfeiffer, & Hildebrand, 2010; Smith, Madec, Coton, & Hymery, 2016).

The dietary consumption of infant population becomes increasingly diverse, moving gradually from breast milk to solid foods or formulae and non-milk based liquids during the second half of their first year (typically around 8–9 months of age) (Daniels et al., 2015). When they grow up, their cereals intake get higher and they are potentially exposed to higher amounts of mycotoxins, which may be hazardous for their health in relation to their reduced body weight compared to adults (Manova & Mladenova, 2009). Subsequently, maximum limits for mycotoxins in baby foods are much lower than the limits set for other cereal products (European Commission, 2006, p. 5). In addition, children and mainly babies are more vulnerable, since their enzymatic activity is not fully completed and therefore their ability to break down chemical compounds is lower and, they are not able to eliminate these molecules (Boon, Bakker, van Klaveren, & van Rossum, 2009; Scheuplein, Charnley, & Dourson, 2002). Moreover, children may be more sensitive to neurotoxic, endocrine disturbance, and immunological toxic effects up to 4 years old (Huybrechts et al., 2011).

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In order to prevent infant exposure to mycotoxins, dietary exposure studies are of great interest. Recent surveys had focused more on regulated mycotoxins (aflatoxins, fumonisins, trichothecenes or zearalenone) than emerging toxins such as enniatins and beauvericin (D'Arco, Fernández-Franzón, Font, Damiani, & Mañes, 2009; González-Osnaya, Soriano, Moltó, & Mañes, 2007; Ware et al., 2017) and very few studies monitoring mycotoxins levels on cereals based infant products (Juan, Raiola, Manes, & Ritieni, 2014; Serrano et al., 2012).

In Tunisia, high amounts of cereals and cereal-based food are consumed but few information is known about eventual mycotoxin contamination especially when it comes to infant population (0–14 years), which represent 23% of the total population (Index mundi, 2016). Based on different expenditure on food, a heterogeneous diet is given to the early age infant ranging from raw cereals (wheat, sorghoum …) and derived products (bsissa, wheat semolina …) to ready to use cereal based packages to both rural and urban populations.

Overall, very few studies have been carried out on the simultaneous natural presence of multi-mycotoxins in cereal-based products in Tunisia (Oueslati et al., 2012; 2014; Juan, Berrada, Manes & Oueslati, 2017). It was also reported that sorghum is highly consumed in Tunisia as infant breakfast ingredient had shown high aflatoxins (AFs) and ochratoxin A (OTA) contamination (Ghali, Hmaissia-Khlifa, Ghorbel, Maaroufi, & Hedili, 2008). To the best of our knowledge, no data are available on the occurrence of mycotoxins contaminating Tunisian infant products based on cereal as the staple food for early aged children.

In the present investigation, an analytical method based on both LC-MS/MS and GC-MS/MS was used to evaluate the presence and the risk exposure of Tunisian infants towards twenty different mycotoxins:deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3AcDON), 15-acetyl-deoxynivalenol (15AcDON), nivalenol (NIV), neosolaniol (NEO), diacetoxyscirpenol (DAS), T-2 and HT-2 toxins, sterigmatocystin (STG), ochratoxin-A (OTA), four aflatoxins (AFB1, AFB2, AFG1, and AFG2), zearalenone (ZON), as well as five emerging mycotoxins: enniatins (ENA, ENA1, ENB and ENB1) and beauvericin (BEA) in cereal and cereal-based products from Tunisia intended for infant consumption.

2. Material and methods

2.1. Chemicals and reagents

Solvents (acetonitrile, hexane and methanol) were purchased from Merck KGaA (Darmstadt, Germany). Anhydrous magnesium sulphate was obtained from Alfa Aesar GmbH & Co. (Karlsruhe, Germany); sodium chloride was purchased from Merck and C18 was purchased from Phenomenex (Torrance, USA). The derivatization reagent composed of BSA (N,O-bis(trimethylsilyl) acetamide) + TMCS (trimethylchlorosilane) + TMSI (*N*-trimethylsilylimidazole) (3:2:3) was purchased from Supelco (Bellefonte, PA). Sodium dihydrogen phosphate and disodium hydrogen phosphate, used to prepare phosphate buffer, were acquired from Panreac Quimica S.L.U. (Barcelona, Spain). The standards of AFB1, AFB2, AFG1, AFG2, OTA, STG, ZON, NIV, DON, 3AcDON, 15AcDON, DAS, NEO, T-2 and HT-2 toxin, BEA, ENA, ENA1, ENB, ENB1, were purchased from Sigma Aldrich (Madrid, Spain).

2.2. Sampling

A set of 117 different cereals and cereal-based products, intended to direct infant population consumption, were purchased from different markets from Tunisia (November 2015 to March 2016) in their commercially available size according to the EU guidance (EU 2006). Samples were composed of rice (n = 6), wheat (n = 20), wheat flour (n = 11), maize (n = 7), sorghum (n = 13), bsissa (n = 18), couscous (n = 8), breakfast cereals (n = 10), biscuits (n = 7), infant cereals (n = 6), baby mix (n = 9), and semolina (n = 2). Bsissa is a mixture of roasted cereals (mainly oat) ground with fenugreek seed, aniseed, cumin and sugar. Rice, wheat, maize and sorghum were available in granulated form. All samples were milled, homogenized thoroughly, stored in polyethylene bags, and maintained under dark and dry conditions before the mycotoxins extraction.

2.3. Extraction of samples

2.3.1. Solid-liquid extraction of samples

Samples extraction was performed according to the previously validated method by Juan, Covarelli, Beccari, Colasante, and Mañes (2016) with slight modifications. First, homogenized and representative portions of 2 g were weighted into 50 mL polypropylene centrifuge tubes and added 10 mL of acetonitrile/water (84:16, v/v). The tubes were shaken using a horizontal shaking device (IKA KS 260 basic) (250 shakes/min) for 1 h. Then, tubes were centrifuged for 5 min at 4500 rpm in 5 °C with Eppendorf Centrifuge 5810R; the supernatant was filtered on Whatman filter paper No. 4 and 5 mL of the supernatant were evaporated to dryness at 38 °C under a gentle stream of nitrogen using a multi-sample Turbo-Vap LV Evaporator (Zymark, Hoptkinton, USA). The dried extract were prepared according the analytical determination technique used, such as in the detailed steps at sections 2.3.2, 2.4 and 2.5.

2.3.2. Derivatization

Before GC-MS/MS analysis, 50 μL of BSA + TMCS + TMSI (3:2:3) was added to the dry extract and left for 30 min to react at room temperature. The derivated sample was diluted to 200 μL with hexane and mixed thoroughly on a vortex for 30 s. Then, to purify the derivate a liquid-liquid extraction with 1 mL of phosphate buffer (60 mM, pH 7) was done and the upper layer was transferred to an autosampler vial for its gas chromatographic analysis.

2.4. GC–MS/MS analysis

The final extract (1 μL) was injected in splitless mode at 250 °C in programmable temperature vaporization (PTV) using an Agilent 7890A GC system coupled with an Agilent 7000A triple quadrupole mass spectrometer with inert electron-impact ion source and an Agilent 7693 autosampler (Agilent Technologies, Palo Alto, CA). The mass spectrometer was operating in electron impact ionization (EI, 70 eV). The source and transfer line temperatures were 230 °C and 280 °C, respectively. The collision gas for MS/MS experiments was nitrogen, and the helium was used as carrier gas at fixed pressure of 20.3 psi, both at 99.999% purity supplied by Carburos Metálicos S.L. (Barcelona, Spain). Data have been acquired and processed using the Agilent MassHunter version B.04.00 software. Analytes have been separated on a HP-5MS 30 m × 0.25 mm x 0.25 μm capillary column. The oven temperature program was initially 80 °C, and the temperature increased to 245 °C progressively at 60 °C/min. After a 3 min hold time, the temperature was increased to 260 °C progressively at 3 °C/min and finally to 270 °C at 10 °C/min and then held for 10 min.

2.5. LC–MS/MS analysis

Before LC–MS/MS analysis, the dry residue was reconstituted to a final volume of 0.5 mL with methanol/water (70:30, v/v) and filtered through a 13mm/0.22 μm nylon filter purchased from Anàlisis Vínicos S.L (Tomelloso, Spain).