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Risk assessment of mycotoxin intake through the consumption of Spanish breadcrumbs

Evaluación del riesgo de ingesta de micotoxinas a través del consumo de pan rallado español

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Abstract: In this work, 80 commercial samples of breadcrumbs, purchased from different supermarkets located in Valencia (Spain), were investigated for the presence of 11 legislated mycotoxins. Only 9 samples evidenced the presence of the legislated mycotoxins. The compounds detected were: ochratoxin A (OTA), zearalenone (ZEA), T-2 toxin (T-2) and HT-2 toxin (HT-2). The incidence of OTA was of 3.75% with concentrations ranging from 1.50 to 6.75 µg/kg. One sample showed a contamination level higher than the maximum legislated in Europe for OTA. Three samples were contaminated with ZEA at concentrations varying from 64.32 to 449.21 µg/kg; all values were higher than the maximum allowed by the legislation. T-2 and HT-2 were detected in two (18.25- 2700.48 µg/kg) and one (147.79 µg/kg) of the 80 samples, respectively. Finally, the estimated dietary intakes (EDIs) of the detected mycotoxins, among the Valencian population, were calculated and the health risk assessment was performed. The calculated EDIs were all below the established tolerable daily intake values (TDI).

Keywords: Mycotoxins, Bread, LC-MS, MS, Estimated daily intake, tolerable daily intake.

Resumen: En este trabajo, 80 muestras comerciales de pan rallado, compradas en diferentes supermercados ubicados en Valencia (España), fueron investigadas por la presencia de 11 micotoxinas legisladas. Sólo 9 muestras evidenciaron la presencia de las micotoxinas legisladas. Los compuestos detectados fueron: ocratoxina A (OTA), zearalenona (ZEA), toxina T-2 (T-2) y toxina HT-2 (HT-2). La incidencia de OTA fue de 3.75% con concentraciones que van desde 1.50 a 6.75 µg / kg. Una muestra mostró un nivel de contaminación más alto que el máximo legislado en Europa para OTA. Tres muestras se contaminaron con ZEA en concentraciones que variaban de 64.32 a 449.21 µg / kg; todos los valores fueron superiores al máximo permitido por la legislación. T-2 y HT-2 se detectaron en dos (18.25-2700,48 µg / kg) y una (147.79 µg / kg) de las 80 muestras, respectivamente. Finalmente, se calcularon las ingestas dietéticas estimadas (EDI) de las micotoxinas detectadas, entre la población valenciana, y se realizó la evaluación del riesgo para la salud. Los EDI calculados estaban todos por debajo de los valores de ingesta diaria tolerable (TDI) establecidos.

Palabras clave: micotoxinas, pan, LC-MS, MS, ingesta diaria estimada, ingesta diaria tolerable.

Introduction

Mycotoxins (Figure 1) are secondary metabolites of fungi that cause toxic and carcinogenic outcomes in humans and animals exposed to them (Wu et al, 2014). The report on the co-occurrence of mycotoxins in food products, either from the same or different fungal species, is becoming more and more frequent (Pereira et al, 2014; Stoev, 2015); their natural co-occurrence is, in fact, an increasing health concern due to the exposure hazard to combined mycotoxins, which could be expected to exert greater toxicity and carcinogenicity than the single mycotoxins (Grenier & Oswald, 2011).

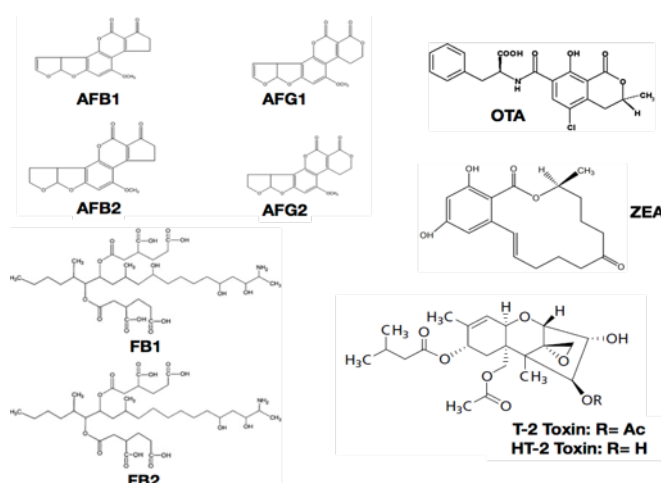


Figure 1

Chemical structures of the legislated mycotoxins analyzed in this study.

People can be intoxicated if they eat either contaminated food or products (such as eggs, meat and milk) from animals that had previously consumed these toxins. In order to reduce the effects of mycotoxin ingestion, the European Union Commission Regulation establishes the maximum levels allowed in certain types of food for the major mycotoxins, such as aflatoxins (AFs), fumonisins (FBs), ochratoxin A (OTA), deoxynivalenol (DON) and zearalenone (ZEA) (European Commission, 2006), and recommends the maximum levels for the sum of T-2 toxin (T-2) and HT-2 toxin (HT-2) (European Commission, 2013).

Food affected by mycotoxin contamination are cereals, nuts, dried fruit, coffee, cocoa, spices, oil seeds, dried peas, beans, several types of fruit (particularly apples), and food products obtained from contaminated raw materials (such as wine and beer) (EFSA, 2013). Mycotoxins are a serious health risk present throughout the entire food chain as they display stability at high temperatures and withstand cooking processes (Bullerman & Bianchini, 2007).

Foodstuffs are very prone to contamination with fungi in the pre- and post-harvest steps and during storage, especially if performed in poor conditions with excessive humidity (Piotrowska et al, 2013). These facts could lead to mycotoxin contamination of foodstuffs, namely cereals and their products, such as breakfast cereals. Over the past few years,

the presence of multiple mycotoxins (mycotoxin mixtures) produced by *Aspergillus* and *Fusarium* genus has been reported in cereals in different countries: AFs, OTA, ZEA and trichothecenes in Spain (Ibáñez-Vea et al, 2011; Montes et al, 2012) and Pakistan (Iqbal et al, 2014); aflatoxin B1 (AFB1) and OTA in Greece (Villa & Markaki, 2009); fumonisins B1, B2, B3 (FB1, FB2, FB3) in Morocco (Mahnine et al, 2012); trichothecenes and ZEA in Italy (Romagnoli et al, 2010); trichothecenes, FBs and ZEA in Canada (Roscoe et al, 2008).

Humans are mainly exposed to mycotoxins by cereals and cereal-derived products. Bread and bread products are a staple food worldwide and, like other perishable products, are susceptible to fungal contamination. Spoilage of bakery products represents a significant source of economic losses to the industry and a potential safety risk due to the mycotoxin production s by diverse molds (mainly *Aspergillus* and *Penicillium*) (Cauvain, 2012; Saranraj & Geetha, 2012; Smith et al, 2004). Bread has a relatively high water activity ($a_w = 0.94-0.97$) with a pH of approximately 6 (Legan, 1993).

The aims of this research were to study the presence of 11 legislated mycotoxins in 80 samples of breadcrumb, to calculate the estimated dietary intakes (EDIs) of the detected mycotoxins in the Valencia population and to assess the health risk comparing the EDIs data with the tolerable daily intake values (TDIs).

Materials and methods

Chemicals and reagents

AFB1, aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), OTA, HT-2 and T-2, DON, ZEA, FB1 and B2, (purity of all mycotoxins > 99%), formic acid (analytical grade, purity > 98%) and ammonium formate (analytical grade, purity $\geq 99.0\%$), were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol (LC-MS grade, purity $\geq 99.9\%$) was purchased from Fisher Scientific (Hudson, NH, USA). Deionized water ($<18\text{M}\Omega$ cm resistivity) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Chromatographic solvents and water were filtered through a $0.45\text{ }\mu\text{m}$ cellulose filter from Scharlau (Barcelona, Spain). All stock solutions were prepared by dissolving 1 mg of the mycotoxin in 1 mL of pure methanol, obtaining a 1 mg/mL solution. These stock solutions were then diluted with pure methanol in order to obtain the appropriated work solutions. All solutions were stored in darkness at -20°C before use.

Bread samples

A total of 80 commercial packages of breadcrumbs were purchased from different supermarkets located in Valencia (Spain) from January to July 2017. These samples were studied and divided into five

categories, according to the Spanish Ministry of Agriculture, Food and Environment (MAGRAMA - Ministerio de Agricultura, Alimentación y Medio Ambiente). The five categories studied were: 23 classical wheat breadcrumbs, 21 breadcrumbs enriched with garlic and parsley, 20 crunchy breadcrumbs, 8 spiced wheat breadcrumbs, and 8 organic wheat breadcrumbs. All products were purchased and analyzed within their shelf-life period. Breadcrumbs of each sample were finely ground, packed in a plastic bag and kept at -20°C until analysis.

Mycotoxin extraction

Extraction of mycotoxins was performed using the method described by Serrano et al. (2013) with some modifications. Three 5 g-aliqouts of each sample of breadcrumb were weighed in 50 mL plastic tubes. Then, 25 mL of methanol were added to each tube and samples were extracted using an Ultra Ika T18 basic ultraturrax (Staufen, Germany) for 3 min. The organic extract was centrifuged at 4000 rpm for 5 min at 5°C and the supernatant was transferred to a flask and evaporated to dryness with a Büchi Rotavapor R-200 (Postfach, Switzerland) at 35°C. The residue was dissolved in 5 mL of methanol, transferred to a 15-mL plastic tube and evaporated to dryness with gaseous nitrogen at 35°C using a multi-sample Turbovap LV Evaporator (Zymark, Hopkinton, USA). Then, the extract was reconstituted in 1 mL of methanol, filtered through a 13mm/0.22 µm filter and transferred to a 1 mL glass vial.

Liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis

The liquid-chromatography (LC) system (Agilent 1200 Chromatograph, Agilent Technologies, Palo Alto, CA, USA) consisted of a binary LC-20AD pump and a SIL-20AC homoeothermic auto sampler. The LC was coupled to a 3200QTRAP mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an ESI interface in positive mode for detection in multiple reactions monitoring (MRM). A CMB-20A controller Analyst Software 1.5.2 was used for data acquisition and processing. The separation of mycotoxins was performed on a Gemini NX C18 column (150×2.0 mm I.D, 3.0 µm, Phenomenex, Palo Alto, CA) at room temperature (20°C). The mobile phase was composed of solvents A (5 mM ammonium formate and 0.1% formic acid in water) and B (5 mM ammonium formate and 0.1% formic acid in methanol) at a flow rate of 0.25 mL/min. The elution gradient was established initially with 10% B, increased to 80% in 1.5 min, then kept constant from 1.5 to 4 min, increased to 90% from 4 to 10 min, increased again to 100% from 10 to 14 min and finally returned to the initial conditions for 10 min. The injection volume was 20 µL. The main MS parameters were optimized and finally set as follows: nebulizer gas (GS1), 55 psi; auxiliary gas (GS2), 50 psi; curtain gas (CUR), 15 psi; capillary temperature, 550 °C; ion spray voltage (IS), 5500 V. Nitrogen was used as the nebulizer, heater,

curtain and collision gas. The precursor-to-product ion transitions and the collision energy used for mycotoxin detection are plotted in Table 1.

Table 1
LC–MS/MS parameters for mycotoxins determination.

Mycotoxin	Retention time (min)	Precursor ion (m/z)	Dwell time (ms)	Product ion (m/z)	CE (eV)
AFB ₁	5.8	313.1	40	241.3	42
				284.9	25
AFB ₂	5.5	315.1	40	259.0	30
				286.9	30
AFG ₁	5.2	329.0	40	243.1	30
				311.1	45
AFG ₂	4.9	331.1	40	313.1	25
				245.1	25
OTA	7.1	404.3	55	102.1	30
				358.1	45
FB ₁	6.5	722.4	55	334.3	42
				352.3	35
FB ₂	7.0	706.4	55	336.2	45
				318.3	40
ZEA	7.2	319.0	55	282.9	15
				301.0	20
T-2	6.8	484.3	55	185.1	15
				215.1	15
HT-2	6.3	442.2	55	215.4	12
				262.8	15
DON	2.2	297.1	170	161.0	10
				249.2	10

Dietary exposure

One of the most important aspects assessing the risk of mycotoxins is to determine the degree of human exposure to these compounds. Therefore, the dietary exposure of Valencia's population to mycotoxins present in breadcrumbs was calculated through EDIs as described below:

$$\text{EDI (ng/kg bw/day)} = \text{mean conc. (ng/kg)} * \text{bread consumption (kg/kg bw/day)}$$

Breadcrumb consumption data were available in the statistical database of the MAGRAMA. Assuming 70 kg as the average body weight (bw) for the population in Valencia, the daily consumption per kg of bw was calculated.

The health risk characterization of each mycotoxin was performed by dividing the previously calculated EDI by the TDI (ng/kg bw/day) of the corresponding mycotoxin (when available), as indicated in the following equation:

$$\% \text{TDI} = (\text{EDI}/\text{TDI}) * 100.$$

Results

Method validation

The method validation included the evaluation of linearity, recovery, repeatability, reproducibility, LODs and matrix effect for each mycotoxin (see Table 2). For the estimation of linearity and matrix effects, the standard calibration curves were carried out for each mycotoxin by plotting the signal intensity versus the mycotoxin concentration. All mycotoxins exhibited good linearity over the working range of the standard solution, the matrix-matched calibration assay and the fortified sample assay. The resulting coefficients of determination (R^2) were always higher than 0.9975. Linearity was evaluated using matrix-matched calibrations in triplicate at concentrations between 5 and 500 $\mu\text{g/Kg}$ for mycotoxins with high sensitivity and between 50 and 2000 $\mu\text{g/Kg}$ for mycotoxins with lower sensitivity. The matrix effect (Table 2) for each mycotoxin was calculated according to the formula defined as the percentage of the matrix - matched calibration slope divided by the slope of the standard calibration curve and multiplied by 100. Recovery analyses were performed in triplicate during 3 consecutive days by spiking blank samples at three different levels (limit of quantitation (LOQ), 2 times LOQ and 10 times LOQ). Spiked samples were left overnight at room temperature to allow solvent evaporation and stabilization of the mycotoxins on the matrix. Results were between 88.3% and 129.2% and relative standard deviation (RSD) was lower than 17%. The values for intra-day repeatability ($n = 3$), expressed as repeatability relative standard deviation (RSDr), ranged from 7.4% to 11.7%; and inter-day reproducibility ($n=5$), expressed as reproducibility relative standard deviation (RSDr), ranged from 8.1% to 17.2% for the same linearity addition values. LODs and LOQs were calculated by analyzing blank samples spiked with the standard mycotoxins (Table 1); they were determined as the lowest concentration of the selected compounds that produce a chromatographic peak at a signal-to-noise ratio (S/N) of 3 and 10 for LOD and LOQ, respectively (Table 2).

Table 2

Validation results in terms of recovery, matrix effect (ME, expressed in %), limits of detection and quantitation (LOD and LOQ, respectively), and linearity (expressed as “r²”) for each mycotoxin.

Mycotoxin	Recovery (%)	ME (%)	LOD (µg/Kg)	LOQ (µg/Kg)	r ²
AFB ₁	128.1	37	0.08	0.27	0.9998
AFB ₂	110.2	29	0.08	0.27	0.9997
AFG ₁	105.2	27	0.16	0.53	0.9998
AFG ₂	108.2	34	0.30	1.00	0.9996
OTA	88.3	102	0.05	0.17	0.9987
FB ₁	105.2	132	50.00	166.67	0.9949
FB ₂	96.2	139	30.00	100.00	0.9975
ZEA	78.2	106	7.80	26.00	0.9991
T-2	129.2	72	1.76	5.87	0.9997
HT-2	118.1	77	4.95	16.50	0.9993
DON	89.2	60	20.50	68.33	0.9989

Occurrence of mycotoxins in breadcrumbs

The occurrence of the aforementioned mycotoxins was determined in 80 units of breadcrumbs. Considering all the analyzed samples, only 9 samples evidenced the presence of the legislated mycotoxins. The compounds detected were OTA, ZEA, T-2 and HT-2. Results of the natural occurrence of mycotoxins are summarized in Table 3. The analyzed samples were not contaminated with AFs, the most important mycotoxins from the toxicological standpoint, which had been found in other cereal-based products due to their stability to environmental conditions and harsh processes, such as baking (Marin et al, 2013).

Table 3.

Incidence and mycotoxin contents in bread crumbs.

Mycotoxins	Positive samples		Samples that exceed the maximum limits		Mean (µg/Kg)	Concentration range (µg/Kg)	IARC classification
	n	Incidence (%)	n	Incidence (%)			
AFB ₁	nd	nd	nd	nd	nd	nd	1
AFB ₂	nd	nd	nd	nd	nd	nd	NC
AFG ₁	nd	nd	nd	nd	nd	nd	NC
AFG ₂	nd	nd	nd	nd	nd	nd	NC
OTA	3	3.75	1	1.25	4.03	1.50-6.57	2B
FB ₁	nd	nd	nd	nd	nd	nd	2B
FB ₂	nd	nd	nd	nd	nd	nd	2B
ZEA	3	3.75	3	3.75	256.76	64.32-449.21	3
T-2	2	2.5	nd	nd	1359.36	18.25-2700.48	NC
HT-2	1	1.25	nd	nd	147.79	147.79	NC
DON	nd	nd	nd	nd	Nd	nd	3

Three samples belonging to the spiced breadcrumb category, were contaminated with OTA with a 3.75% incidence. The average concentration found was 4.03 µg/kg, with a contamination range varying

from 1.50 to 6.57 $\mu\text{g/kg}$. Only one sample displayed an OTA content (6.57 $\mu\text{g/kg}$) higher than the maximum allowed level by the EU legislation (3 $\mu\text{g/kg}$; European Commission, 2006).

Three of the 80 analyzed samples, were contaminated with ZEA. These positive samples were all crunchy breadcrumbs, and had a 3.75% overall incidence. The three samples showed a contamination level above the limit enforced by the European legislation (50 $\mu\text{g/kg}$; European Commission, 2006); the detected contamination range varied from 64.32 to 449.21 $\mu\text{g/kg}$, with a mean contamination level of 256.76 $\mu\text{g/kg}$.

Two of the 80 analyzed samples, were contaminated with the T-2 toxin. The samples positive to this mycotoxin were: one sample of the classical wheat breadcrumbs and one sample of the product containing garlic and parsley, which also evidenced the presence of the HT-2 toxin. The detected contamination incidence was 2.5% and 1.25% for T2 and HT-2, respectively. The sample contaminated with the HT-2 toxin displayed a contamination level of 147.79 $\mu\text{g/kg}$, whereas the T-2 toxin contamination level ranged from 18.25 to 2700.48 $\mu\text{g/kg}$, with a mean contamination level of 1359.36 $\mu\text{g/kg}$.

Estimation of the daily intake of mycotoxins in breadcrumbs

The EDIs and health risk characterization (% of relevant TDI) of the mycotoxins detected in the analyzed samples are shown on Table 4.

Table 4
Mycotoxins' exposure through estimated daily intake (EDI) parameter and risk assessment of the Valencia population through the breadcrumb consumption.

Mycotoxins	EDI ($\mu\text{g/kg bw/day}$)	TDI ($\mu\text{g/kg bw/day}$)	%EDI/TDI
HT-2	6.48×10^{-5}	0.06	0.10
OTA	3.91×10^{-6}	0.02	0.03
T-2	1.3×10^{-3}	0.06	2.16
ZEA	7.22×10^{-4}	0.20	0.36

The ZEA EDIs in the breadcrumbs was 7.22×10^{-5} $\mu\text{g/kg bw/day}$ and, considering that the ZEA TDI was 0.2 $\mu\text{g/kg bw/day}$, the percentage of the EDI on the TDI was 0.36%. Saladino et al. (2017) calculated the EDIs for ZEA contained in different bread loaf samples, which varied from 2.380 to 2.923 ng/kg bw/day considering the lower bound (LB) and the upper bound (UB) scenarios, respectively. The %TDI was 0.952% for LB and 1.169% for UB. EDI values calculated for ZEA through the consumption of different commodities in Catalonia ranged between 0.3 and 0.5 ng/kg bw/day (Cano-Sancho et al, 2012), which is 5-6 times lower than the results found in the present study. Moreover, Aldana et al (2014) found EDIs of 0.049 and 0.090 $\mu\text{g/kg bw/day}$ in Portugal and

the Netherlands, respectively, through the consumption of contaminated wheat flour, which is the main ingredient of bread.

The EDI calculated in this study for the T-2 toxin was $1.30 \times 10^{-3} \mu\text{g}/\text{kg bw}/\text{day}$, and, considering that this mycotoxin has a TDI of $0.06 \mu\text{g}/\text{kg bw}/\text{day}$, the risk calculated for the T-2 toxin was 2.16% (as %EDI on TDI). Bearing in mind the risk assessment calculated for the Valencian population exposed to OTA intake through breadcrumb consumption, the EDI calculated for this mycotoxin was $3.91 \times 10^{-6} \mu\text{g}/\text{kg bw}/\text{day}$ and considering that this compound has a TDI of $0.02 \mu\text{g}/\text{kg bw}/\text{day}$, the %EDI on the TDI was 0.03%. The result obtained for the risk assessment related to the HT-2 toxin intake; through the EDI estimation was of $6.48 \times 10^{-5} \mu\text{g}/\text{kg bw}/\text{day}$. Comparing this data with the HT-2 TDI ($0.06 \mu\text{g}/\text{kg bw}/\text{day}$), the %EDI on the TDI data was 0.10 $\mu\text{g}/\text{kg bw}/\text{day}$. Considering a general evaluation on the risk assessment related with the mycotoxins detected in the analyzed samples, the mycotoxin with the lowest toxicological risk was OTA, as it had the lowest %EDI on the TDI (0.026%), while the higher risk was observed for the T-2 mycotoxin where the %EDI on the TDI was 2.16%.

Discussion

Occurrence of mycotoxins in breadcrumbs

This article is the first where the legislated mycotoxins were determined by LC-MS/MS in breadcrumbs of Valencian origin. The presence of the legislated mycotoxins in other bread products, has been evaluated by many authors.

Recently, Saladino et al. (2017) studied the mycotoxin contamination of 80 commercial samples of bread loaves purchased from different supermarkets located in Valencia (Spain). Results showed that samples were contaminated with AFs, ZEA and Enniatins (ENs) with a frequency of 20, 96, and 65% respectively. AFB1, AFB2 and AFG1 were detected with concentrations ranging from 0.5 to $7.1 \mu\text{g}/\text{kg}$. The samples contaminated with AFB1 showed values exceeding the maximum limit allowed in the EU. The sum of AFs also exceeded the maximum permitted limit in 6 samples. ENs contamination data ranged from 0.2 to $54 \mu\text{g}/\text{kg}$, and enniatin B (ENB) was the most prevalent one. ZEA values varied from 27 to $905 \mu\text{g}/\text{kg}$, and 30% of the contaminated samples were above the limits enforced by the EU.

Bol et al. (2016) studied the processing effect on mycotoxins levels and the exposure to ZEA, OTA and AFB1 through the consumption of pasta and bakery products. The higher reduction percentage of mycotoxins was observed in cake production (95, 90 and 70% for ZEA, OTA and AFB1, respectively). Bread and biscuit showed similar reduction in mycotoxins levels (89 and 90% for ZEA; 80 and 85% for OTA; 36 and 40% for AFB1, respectively), while pasta displayed the lowest reduction in the mycotoxins levels (75, 65 and 10% for ZEA, OTA and AFB1, respectively).

Iqbal et al. (2014) analyzed AFs, OTA and ZEA in breakfast cereals. Their results showed that 41% of the samples were positive for the AFs presence, being twofold higher than the one obtained in the present study. The authors also reported that 16% and 8% of the samples presented AFB1 and total AFs levels, respectively, above the limits enforced by the European legislation. Moreover, the co-occurrence and risk assessment of different mycotoxins in cereals and cereal-based products from Mediterranean area was also evaluated (Serrano, Font, Ruiz & Ferrer, 2012), where 10.2% of the samples were contaminated with AFs at much higher concentrations (4.2-66.7 µg/kg) than the contamination level found in our study.

Estimation of the daily intake of mycotoxins in breadcrumbs

Bol et al. (2016) defined that the consumption of Brazilian bakery products (such as bread, cake, biscuit and pasta) could represent 12.6% of the maximum tolerable daily intake of ZEA and 30.5% of the tolerable weekly intake of OTA. The margin of exposure value related to AFB1 exposure was 24.6. The exposure to ZEA and OTA through pasta consumption showed the highest percentage of tolerable intake of these mycotoxins established by Joint FAO/WHO Expert Committee on Food Additives (JECFA) (7.8% of provisional maximum tolerable daily intake (PMTDI) for ZEA and 17% of provisional tolerable weekly intake (PTWI) for OTA). In this hypothetical situation, bread may be the second most important source of exposure to ZEA and OTA in the diet, contributing to 3.9% of PMTDI for ZEA and 11% of PTWI for OTA. Biscuit and cake had similar percentage of contribution for PMTDI of ZEA (0.5% for both biscuit and cake) and PTWI of OTA (1.1% for biscuit and 1.4% for cake).

Coronel et al. (2012) evaluated the OTA contamination in composite samples of cereal-based baby foods, beer, breakfast cereals (corn, rice and wheat-based, loaf bread, peanuts and pistachios, collected in hypermarkets and supermarkets from 12 cities in the Spanish region of Catalonia. Consumption data for the selected foodstuffs were collected by means of a food-frequency questionnaire. The studied population was grouped by age in infants, children, adolescents and adults; exposure to OTA through the abovementioned foodstuffs, as well as through wine and coffee, was assessed. Exposure assessment was performed by deterministic and probabilistic modeling of the contamination and consumption data. The median estimated daily intake of OTA through the aforementioned foodstuffs in each age group, were below the latest provisional tolerable daily intakes (PTDIs) of 17 and 14 ng/kg bw/day recommended by the European Food Safety Authority (EFSA) in 2006 and by JECFA in 2007, respectively, ranging from 1% and 2% of those PTDIs values in adolescents and children, to 3% and 11% in adults and infants.

Rodriguez-Carrasco et al. (2013) studied the quantitation of mycotoxins in cereal-based food, highly consumed by different age

population of Valencian origin. Cereal-based samples classified as wheat-, maize- and rice-based, were subjected to the evaluation of mycotoxin occurrence (patulin, DON, 3-acetyl-deoxynivalenol, fusarenon-X, diacetoxyscirpenol, nivalenol, neosolaniol, HT-2, T-2 and ZEA) by gas chromatography–tandem mass spectrometry. Intakes were calculated for average consumers in adults, children and infants and compared with the TDI. Data obtained were used to estimate the potential exposure levels. In fact, 65.4% of the samples were contaminated with at least one mycotoxin and 15.7% of the analyzed samples showed co-occurrence of mycotoxin. The dietary exposure to HT-2 and T-2 toxins was estimated as 0.010 and 0.086 $\mu\text{g/kg bw/day}$, amounting to 10% and 86% of the TDI for adults and infants, respectively.

Conclusions

To our knowledge, this is the first study on the presence of legislated mycotoxins in breadcrumbs commercialized in Valencia. Only 9 of the 80 samples analyzed were contaminated by mycotoxins: three by OTA, three by ZEA, two by T-2 and one by HT-2. One sample contaminated with OTA and all the breadcrumbs contaminated by ZEA exceeded the maximum limits permitted by the EU legislation. Although the presence of mycotoxins was in some cases above the limits of European legislation, samples were all free from AFs which are the most important mycotoxins from the toxicological standpoint and the calculated EDIs were all lower than the TDI value. However, to have a complete view of the risk assessment, it should be considered the total mycotoxins intake deriving from the whole dietary regime. Further studies are thus necessary to analyze the levels of these mycotoxins in several other foods.

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