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Mycotoxins in cereal-based infant foods marketed in China: Occurrence and risk assessment

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ABSTRACT

Infants are considered as the most vulnerable subgroup susceptible to mycotoxins in food. A total of 820 cerealbased infant foods marketed in China were screened for 20 mycotoxins using an ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method. Low levels of 12 mycotoxins were detected including deoxynivalenol (DON), tenuazonic acid (TeA), enniatin A (ENA), zearalenone (ZEN), enniatin B1 (ENB1), alternariol (AOH), enniatin B (ENB), alternariol nonomethyl ether (AME), enniatin A1 (ENA1), fumonisin B1 (FB1), ochratoxin A (OTA), and fumonisin B2 (FB2) with the incidence rate of 55.7, 45.1, 13.9, 8.2, 6.6, 5.6, 4.8, 4.3, 3.9, 3.7, 1.1, and 0.7% of samples, respectively. One or more mycotoxins were found in 73.7% of all samples, 71.2% of processed infant foods (infant cereal, infant cracker, and infant noodle), and 82.3% in common cereals (wheat flour, rice, and millet) for infant consumption. Wheat-based infant foods were contaminated with more varieties of mycotoxins than those rice-based infant foods. A deterministic approach was used to evaluate dietary risk assessment through cereal-based food consumption for the average and heavy infant food intakes was considered to be acceptable (Hazard quotient <1). However, the estimated dietary exposure of AME and AOH exceeded the threshold of toxicological concern of 0.0025 μ g/kg b.w./day, which confirmed the need for further study to assess the potential health risks for infants.

1. Introduction

Mycotoxins are secondary metabolites of certain types of filamentous fungi, such as *Aspergillus, Penicillium, Fusarium*, and *Alternaria* (Yang et al., 2020), which are considered a global public health threat. To date, over 400 mycotoxins have been reported worldwide. The important groups of mycotoxins that include aflatoxins (AFB1, AFB2, AFG1, AFG2 and AFM1), ochratoxin A (OTA), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FB1, FB2), T-2, and HT-2 toxins (HT-2) are particularly concerned and strictly regulated via government legislations (Binder, Tan, Chin, Handi, & Richard, 2007). Among mycotoxins, AFB1 is classified as a human carcinogen (Group 1) by the International Agency for Research on Cancer. AFM1, OTA, and fumonisins are classified as possible carcinogens to humans (Group 2B). DON, ZEN, T-2, and HT-2 are classified as non-carcinogenic to humans (Group 3) (Yang et al., 2020). In the last decade, a group of emerging mycotoxins such as *Alternaria* toxins including alternariol (AOH), alternariol nonomethyl ether (AME), tenuazonic acid (TeA) and enniatins (ENs) (ENA, ENA1, ENB, and ENB1), which have been found in a wide range of raw commodities and derived products as food contaminants, have gained increasing attention (Metzler, Pfeiffer, & Hildebrand, 2010; Smith, Madec, Coton, & Hymery, 2016). Therefore, monitoring the occurrence of mycotoxins in foods, especially the emerging mycotoxins with limited toxicity data, is highly important to ensure human health.

Grains are prone to contamination with mycotoxins under appropriate conditions of temperature and humidity (Khaneghah, Fakhri, Gahruie, Niakousari, & Sant'Ana, 2019). For infants, cereal foods are an important source of their dietary diet. In previous studies, natural mycotoxins have been evaluated in a variety of infant foods. OTA, DON, HT-2, ENB, ENB1, and ENA1 were found in amounts of 20%, 21%, 3%, 15%, 1%, and 4% of the analyzed infant foods from the Italian market, respectively (Juan, Raiola, Manes, & Ritieni, 2014). The occurrences of AFM1 (33%), OTA (31%), DON (27%), AFB1 (22%), FB2 (10%), ZEN

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Abbrevi	ations	AOH	Alternariol
		TeA	Tenuazonic acid
AFB1	Aflatoxin B1	ENs	Enniatins
AFB2	Aflatoxin B2	ENA	Enniatin A
AFG1	Aflatoxin G1	ENA1	Enniatin A1
AFG2	Aflatoxin G2	ENB	Enniatin B
AFM1	Aflatoxin M1	ENB1	Enniatin B1
OTA	Ochratoxin A	HBGV	Health-based guidance values
ZEN	Zearalenone	MRM	Multiple reaction monitoring
DON	Deoxynivalenol	ESI	Electrospray ionization
3-ADON	3-acetyl-deoxynivalenol	LOD	Limit of detection
15-ADO	N 15-acetyl-deoxynivalenol	LOQ	Limit of quantification
FB	Fumoinisins	EDI	Estimated daily intake
FB1	Fumonisin B1	HQ	Hazard quotient
FB2	Fumonisin B2	TDI	Tolerable daily intake
HT-2	HT-2 Toxin	TTC	Threshold of toxicological concern
T-2	T-2 Toxin	UPLC-M	S/MS Ultra-high performance liquid chromatography-
AME	Alternariol monomethyl ether		tandem mass spectrometry

(4%), and T-2 toxin (2%) were found in commercial formula milk and cereal-based baby foods in Qatar (Hassan et al., 2018). Low levels of AFB1, ZEN, DON, and FB1 were detected in infant foods with concentration levels of 0.4, 2.3, 131, 39 μ g/kg, respectively, in Europe (Braun, Eiser, Puntscher, Marko, & Warth, 2021).

Although previous studies have reported the occurrence of mycotoxins in infant foods, only few studies have conducted assessment of dietary risk of mycotoxins to infants (Vin et al., 2020). The 90th percentile chronic dietary exposure to nivalenol, patulin, fumonisins, and zearalenone for children was less than 40% of the health-based guidance values (HBGV) considered relevant for children. Kirimer, Turksoy, and Kabak (2020) reported that the chronic mean and 95th percentile dietary exposure to DON and FBs for infants through cereal-based infant food consumption were within the threshold level of 1 μ g/kg b.w./day, indicating an acceptable dietary risk. Braun et al. (2021) reported that the dietary exposure of DON was 1.6 μ g/kg b. w./day, which exceeded the guidance value, indicating potential dietary health risks for infants.

Evaluation of the occurrence of mycotoxins in infant foods and the estimation of dietary exposure to mycotoxins for infants have been evaluated in different countries. However, to the best of our knowledge, published data are limited on the occurrence of natural mycotoxins, especially the presence of the emerging mycotoxins (e.g., *Alternaria* toxins and enniatins) in infant foods marketed in China. In addition, dietary exposure of mycotoxins estimated for infants is scarce in China. The present study was aimed to evaluate the occurrence of mycotoxins in cereal-based infant foods marketed in China and estimate the dietary exposure of detected mycotoxins for Chinese infants.

2. Materials and methods

2.1. Chemicals, reagents, and standards

Acetonitrile and methanol were high-performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany). Formic acid (purity >99%) and acetic acid were HPLC grade (Tedia, Fairfield, USA). Ammonium acetate (\geq 99.0%) and ammonium formate (\geq 99.0%) were analytical grade and were obtained from Huadong Medicine (Hangzhou, China). Purified water was obtained using a Millipore Milli-Q apparatus (Massachusetts, USA).

Standards of AFB1, AFB2, AFG1, AFG2, OTA, DON, 3-ADON, 15-ADON, ZEN, HT-2, T-2, FB1, FB2, ENA, ENA1, ENB, ENB1, AME, AOH, and TeA were obtained from Romer labs (Union City, Missouri, U.S.A.). Standard stock solution of each analyte was prepared at concentration of 100 mg/L in acetonitrile, and multi-mycotoxin working standard solutions of 1 mg/L were prepared before preparing series of working standard solution. One series of mixed multi-mycotoxin working standard solution was prepared in methanol-water (1:1, v/v) at concentrations of 0.1, 1, 5, 10, 20, 50, 100, and 200 μ g/L, and the other series of mixed multi-mycotoxin working standard solutions were prepared in blank matrix extract.

2.2. Sample collection

A total of 820 samples from 639 processed infant foods (133 infant cereals, 229 infant noodles, and 277 infant crackers) and 181 common cereals for infants (58 rice, 69 millets, 54 wheat flour) were randomly collected from maternal- and child-focused grocery stores, supermarkets, and e-commerce stores in China. These samples originated from 18 provinces and cities in China, including Shandong, Henan, Hebei, Shanghai, Jiangsu, Zhejiang, Jiangxi, Fujian, Guangdong, Guangxi, Taiwan, Hunan, Hubei, Jilin, Heilongjiang, Shanxi, Ningxia, and Neimenggu. Among these samples, 10 infant cracker samples were from Malaysia, Japan, Singapore, Thailand, the UK, Italy, Germany, and Poland, and 6 infant cereal samples were from Germany. The samples were produced by 182 suppliers. Rice powder in infant cereals made up 83%-95% of the samples. For infant noodles, the content of wheat flour accounted for 98% of the samples. For infant crackers, the content of wheat flour accounted for 49%-62% of the samples. In addition to the main ingredient of rice powder or wheat flour in cereal-based infant foods produced in China, a variety of ingredients were added to increase flavor, including mixed fruit/vegetable powder, mixed nuts powder, egg yolk powder, whole milk powder, whey protein powder, or beef powder. As for raw cereals, different forms of packing and bulk rice, millets, and wheat flour were purchased.

In this study, solid form samples (infant cereals, infant noodles, infant crackers, rice, and millet) were ground to powder form with a grinding mill, passed through a 20-mm round hole sieve, and mixed together. Special care was taken for cracker sample preparation by using a reducer device to prevent samples from overheating during processing. A quadratic sampling method (GB 5491–1985) was applied in lab. Analytical samples stored in plastic cans in a -20 °C freezer prior to analysis.

2.3. Analytical method

A multi-mycotoxin determination method was developed for 20 mycotoxins including AFB1, AFB2, AFG1, AFG2, OTA, DON, 3-ADON,

15-ADON, ZEN, HT-2, T-2, FB1, FB2, ENA, ENA1, ENB, ENB1, AME, AOH, and TeA in cereal-based infant foods based on the method reported by Soleimany, Jinap, and Abas (2012) with some modifications. Briefly, a representative sample (5 g) with 0.5 g NaCl was extracted with 25 mL acetonitrile:water:formic acid (79:20:1, v/v) solution. The extraction process was as the following: samples were vortexed vigorously for 5 min by a multi-tube vortexer (DuraSafer (Beijing) Technology Ltd., China), kept standing for 30 min, again vortexed for 5 min by the multi-tube vortexer, and then sonicated for 30 min at room temperature with an ultrasonic cleaning machine (Kunshan Ultrasonic Instrument Co., Ltd, China). The extraction mixture was centrifuged at 8500 rpm for 5 min (Sorvall ST8R, Thermo Scientific, USA). After centrifugation, 5 mL of the supernatant extract was evaporated to dryness under a nitrogen stream (MTN-2800W, AutoScience, Tianjin, China). The residue was dissolved in 1 mL of methanol:water mixture (1:1, v/v) and vortexed for approximately 30 s. The solution was transferred into a 1.5 mL centrifuge tube and centrifuged at 12,000 rpm for 3 min. The supernatant was then passed through a 0.22 μ m nylon filter and analyzed using an ultra-high-performance liquid chromatograph coupled with mass spectrometry (UPLC-MS/MS). Recovery analysis was conducted using three different concentrations of fortified blank samples following the above procedures.

2.4. UPLC-MS/MS analysis

A UPLC-MS/MS system equipped with an AB Sciex 6500 Qtrap tandem mass spectrometer (Redwood City, CA, USA), a Shimadzu LC-30AD quaternary pump (Tokyo, Japan), SIL-30AC autosampler, and CTO-20AC column oven was used for the detection and quantification of AFB1, AFB2, AFG1, AFG2, OTA, DON, 3-ADON, 15-ADON, ZEN, HT-2, T-2, FB1, FB2, ENA, ENA1, ENB, ENB1, AME, AOH, and TeA multimycotoxins. A Waters ACQUITY BEH C_{18} guard cartridge (2.1 \times 5 mm, 1.7 $\mu\text{m})$ and a Waters ACQUITY BEH C_{18} analytical column (2.1 \times 100 mm, 1.7 μ m) were used for separation. The mobile phase consisted of methanol (A) and 5 mmol/L ammonium acetate (B). A binary gradient was set as in the following: 0–1.0 min, 95% B; 1.0–6.0 min, 95%–5% B; 6.0-8.0 min, 5% B; 8.0-8.1 min, 5%-95% B; and 8.1-10.0 min, 95% B. The flow rate was 0.3 mL/min and the injection volume was 5 $\mu L.$ Multiple reaction monitoring (MRM) was applied for the MS/MS in both positive and negative electrospray ionization (ESI+/-) mode for the multi-mycotoxin detection. The optimized operation conditions were set as in the following: ion spray voltage at +5500/-4500 V, source temperature at 500 °C, curtain gas pressure at 35 psi, GS1 pressure at 50 psi, and GS2 pressure at 55 psi for both modes.

2.5. Quality control

In this study, instruments' performance and reproducibility were measured by injecting quality control samples in triplicates at two levels of reference standards in solvent at the beginning and end of each measurement. Through comparing the peak area ratios, evaluating the relative deviation of the retention time within $\pm 2.5\%$, and the relative abundance ratio within $\pm 25\%$ between the quantification and confirmation transitions (Commission Decision 2002/657/EC), positive findings were confirmed. Two spiked samples were analyzed with each type of sample batch.

2.6. Risk assessment

Exposure analysis and risk assessment are essential to quantify the dietary risk of chemicals present in food for humans. Due to its simplicity, worldwide use, and acceptance, the deterministic approach with reference to Devriese et al. (2005) was used to evaluate dietary exposure of mycotoxins for infants through cereal-based infant food consumption. Here, we assumed the middle bound worst-case scenario by considering the LOD/2 as the quantified maximum concentrations for

analytes which were not detected in samples. The estimated daily intakes (EDIs) and the hazard quotient (HQ) values of mycotoxins were calculated by the following equations: estimated daily intake = (concentration of analytes in food × food intake)/body weight and HQ (%) = (estimated daily intakes/reference values) × 100% (IPCS, 2009).

Consumption data of cereal-based infant foods were from the crosssectional survey on individual dietary consumption of children under 3 years old which was conducted by the National Center for Food Safety Risk Assessment in 2015. A 24-h recall method was applied for the survey by using a multistage, stratified cluster random sampling method over non-consecutive 3 days (the interval of two surveys was more than 3 days). During survey, consumption data of 20,172 infants under 3 years of age were collected by interviewing the infants' parents or primary caregivers (Wang et al., 2019). Here, the daily cereal-based infant consumption in China was estimated to be 0.046 kg/day for average infant consumers and 0.129 kg/day for heavy infant consumers (97.5th percentile). The mean body weight of infants in China is 11 kg (Wang et al., 2019).

2.7. Statistical analysis

When the levels of mycotoxin detected in samples were above the LOQ, the samples were considered positive, while samples with the levels of contamination lower than LOQ were considered negative. During dietary risk assessment, non-detection was considered half the LOD (LOD/2) for the levels of mycotoxins below the LOQ (European Food Safety Authority EFSA, 2010). Multiquant 3.0 (Sciex, Framingham, MA, USA) was used for chromatographic data analysis and processing. Dietary risk assessment and related calculations to this study were performed using Microsoft Office Excel 2010.

3. Results and discussion

3.1. Method validation

The linearity, accuracy, matrix effects, limit of detection (LOD), and limit of quantification (LOQ) were validated for the developed method under the guidance from SANCO/12495/2011(SANCO, 2011). As for UPLC–MS/MS analysis, the optimized MS/MS acquisition parameters are shown in Table 1. The validation results of the developed method are shown in Table 2. The matrix effect was evaluated by the slope ratio of the matrix-matched calibration curve to the solvent standard calibration curve. The results showed that the slope ratio ranged from 0.4 to 1.3, demonstrating the existence of matrix effect, so the matrix-matched multi-external standards was used for qualitative and quantitative analysis. The results from the method validation verified the feasibility of the developed method.

3.2. Mycotoxins in cereal-based infant foods

A total of 820 cereal-based infant foods were screened for 20 types of mycotoxins. Overall, one or more mycotoxins were found in 73.7% (604/820) of all samples, 71.2% of processed infant foods (infant cereal, infant cracker, and infant noodle), and 82.3% in common cereals (wheat flour, rice, and millet) for infant consumption. The detection rate of samples with mycotoxin contamination was 100.0%, 97.8%, 94.2%, 80.9%, 51.7%, and 5.3% in wheat flour, infant noodle, millet, infant cracker, and infant cereal, and rice samples, respectively. Among 20 mycotoxins analyzed, 12 types of mycotoxins were detected, including *Fusarium* mycotoxins (DON, ZEN, FB1, FB2, ENA, ENA1, ENB, ENB1), OTA, and *Alternaria* toxins (AME, AOH, TeA). The incidence rates of mycotoxins followed the trend in the following: DON (55.7%) > TeA (45.1%) > ENA (13.9%) > ZEN (8.2%) > ENB1 (6.6%) > AOH (5.6%) > ENB (4.8%) > AME (4.3%) > ENA1(3.9%) > FB1 (3.7%) > OTA (1.1%) > FB2 (0.7%).

The MS/MS acquisition parameters that were used for the analysis of mycotoxins in cereal-based infant foods.

Analytes	Precursor ion (m/z)	Product ions (m/z)	Declustering potential (eV)	Collision energy (eV)	ESI	Retention time (min)
AFB1	313.1	241.1 ^a	120	46.8	+	5.69
		269.0	120	39.2		
AFB2	315.1	287.0 ^a	120	38.7	+	5.58
		259.1	120	38.7		
AFG1	329.1	243.0 ^a	120	35.0	+	5.45
		215.0	120	42.8		
AFG2	331	245.1 ^a	120	39.0	+	5.31
		217.0	120	47.1		
OTA	402.1	358.3 ^a	-65	-25.0	_	6.05
		211.1	-65	-34.0		
DON	295.1	265.1 ^a	-32	-15.0	_	4.20
		138.1	-32	-23.0		
3-ADON	339.2	203.2 ^a	40	18.9	+	5.19
		175.1	40	25.9		
15-ADON	339.3	137.1 ^a	40	23.0	+	5.20
		321.3	80	12.0		
ZEN	317.2	175.0 ^a	-153	-32.0	-	6.61
		131.0	-153	-39.0		
HT-2	447.2	285.0 ^a	60	26.0	+	6.20
		345.1	60	22.9		
T-2	489.2	245.2 ^a	110	34.6	+	6.42
		327.1	110	31.0		
FB1	722.3	334.2 ^a	40	53.0	+	5.74
		352.2	40	48.0		
FB2	706.2	336.2 ^ª	40	46.5	+	6.35
		318.2	40	49.0		
ENA	699.4	682.3 ^a	40	23.5	+	7.67
		210.1	40	38.1		
ENA1	668.6	210.2 ^a	125	29.2	+	7.55
		541.2	125	30.2		
ENB	657.4	640.3 ^a	40	24.2	+	7.36
		196.1	40	39.7		
ENB1	654.7	196.2 ^a	100	35.6	+	7.46
		210.2	100	30.2		
AME	271.2	256.2^{a}	-100	-28.0	_	6.80
		228.2	-100	-42.0		
AOH	257	213.2ª	-120	-32.0	_	6.08
		147.2	-120	-42.0		
TeA	196	139.0 ^a	-50	-28.0	_	3.91
		112.0	-50	-40.0		
		112.0	-50	-40.0		

^a Quantification ion.

Table 2

Method performance characteristics of 20 mycotoxins analyzed in cereal-based infant foods.

Analytes	Limit of Quantification (LOQ)	Calibration range	range Average recoveries (Mean \pm RSD) ^a						
			Infant cereal	Infant cracker	Infant noodle	Wheat flour	Rice	Millet	
	[µg/kg]	[µg/L]	[%]	[%]	[%]	[%]	[%]	[%]	
AFB1	0.1	0.1-200	91.7 ± 8.2	83.7 ± 3.3	84.9 ± 4.1	100.5 ± 7.6	$\textbf{85.7} \pm \textbf{4.3}$	$\textbf{91.9} \pm \textbf{8.2}$	
AFB2	0.1	0.1-200	110.5 ± 4.2	114.3 ± 4.5	101.8 ± 10.2	109.3 ± 6.1	113.7 ± 5.9	107.5 ± 6.5	
AFG1	0.1	0.1-200	$\textbf{92.9} \pm \textbf{3.8}$	$\textbf{83.0} \pm \textbf{8.2}$	$\textbf{87.0} \pm \textbf{6.4}$	92.9 ± 6.6	$\textbf{85.4} \pm \textbf{4.4}$	$\textbf{94.1} \pm \textbf{7.8}$	
AFG2	0.3	0.3-200	116.5 ± 8.3	107.9 ± 12.6	93.0 ± 6.3	107.1 ± 9.3	111.6 ± 8.8	103.3 ± 10.7	
OTA	0.1	0.1-200	107.9 ± 2.8	84.3 ± 3.4	88.6 ± 3.5	100.6 ± 3.1	105.3 ± 2.1	$\textbf{70.2} \pm \textbf{3.2}$	
DON	3.0	3.0-200	93.3 ± 4.7	$\textbf{98.1} \pm \textbf{5.8}$	92.7 ± 5.9	99.6 ± 4.2	95.0 ± 6.3	92.1 ± 10.5	
3-ADON	3.0	3.0-200	108.8 ± 9.4	110.2 ± 10.6	99.6 ± 11.8	110.7 ± 10.9	94.9 ± 14.3	101.9 ± 16	
15-ADON	3.0	3.0-200	114.3 ± 8.9	117.1 ± 14.7	100.3 ± 9.6	$\textbf{86.2} \pm \textbf{14.2}$	104.2 ± 7.7	109.7 ± 10.3	
ZEN	1.0	1.0-200	106.4 ± 1.6	80.9 ± 2.5	87.3 ± 1.0	95.2 ± 1.9	99.5 ± 2.9	61.9 ± 3.7	
FB1	1.0	1.0-200	91.7 ± 7.1	$\textbf{76.6} \pm \textbf{11.4}$	$\textbf{80.9} \pm \textbf{14.1}$	$\textbf{84.3} \pm \textbf{14.9}$	111.3 ± 13.2	93.1 ± 10.0	
FB2	1.0	1.0-200	90.7 ± 9.9	$\textbf{74.8} \pm \textbf{13.5}$	$\textbf{85.9} \pm \textbf{10.8}$	$\textbf{92.2} \pm \textbf{10.7}$	118.3 ± 5.1	$\textbf{94.2} \pm \textbf{14.3}$	
HT-2	1.0	1.0-200	109.6 ± 13.3	$\textbf{85.4} \pm \textbf{9.3}$	93.2 ± 12.4	110.7 ± 14.3	112.4 ± 14.7	114.1 ± 8.1	
T-2	1.0	1.0 - 200	111.0 ± 5.4	103.1 ± 7.8	100.0 ± 6.5	107.3 ± 7.2	111.7 ± 4.5	$\textbf{99.9} \pm \textbf{9.9}$	
ENA	0.8	0.8-200	53.4 ± 5.8	$\textbf{58.5} \pm \textbf{3.7}$	$\textbf{78.2} \pm \textbf{4.8}$	57.3 ± 3.0	51.6 ± 5.4	$\textbf{30.2} \pm \textbf{5.2}$	
ENA1	0.8	0.8-200	$\textbf{70.0} \pm \textbf{112.3}$	69.9 ± 12.4	79.6 ± 11.7	72.6 ± 10.9	70.0 ± 11.3	$\textbf{48.8} \pm \textbf{12.6}$	
ENB	0.8	0.8-200	$\textbf{95.2} \pm \textbf{4.3}$	$\textbf{86.9} \pm \textbf{4.2}$	97.7 ± 3.6	93.4 ± 4.5	93.0 ± 2.8	$\textbf{35.9} \pm \textbf{5.2}$	
ENB1	0.8	0.8-200	80.6 ± 12.9	99.5 ± 10.0	$\textbf{86.6} \pm \textbf{14.5}$	$\textbf{85.4} \pm \textbf{10.7}$	89.7 ± 9.5	$\textbf{32.2} \pm \textbf{13.8}$	
AME	1.0	1.0-200	98.5 ± 2.4	60.1 ± 3.7	64.0 ± 3.3	61.8 ± 1.6	$\textbf{85.2} \pm \textbf{1.9}$	60.2 ± 3.3	
AOH	1.0	1.0-200	101.9 ± 3.1	$\textbf{92.4} \pm \textbf{2.1}$	82.3 ± 3.1	100.4 ± 3.8	104.7 ± 3.6	$\textbf{87.0} \pm \textbf{3.8}$	
TeA	3.0	3.0-200	$\textbf{70.5} \pm \textbf{13.2}$	90.7 ± 5.3	97.3 ± 4.0	97.8 ± 4.0	$\textbf{96.5} \pm \textbf{8.0}$	100.2 ± 12.5	

 a Average recoveries (Mean \pm RSD) obtained from each type of cereal-based samples which was spiked with mycotoxins at 5, 10, and 100 μ g/kg.

3.2.1. Fusarium toxins

Fusarium toxins are commonly present in cereals and their related products. In this study, Fusarium toxins including DON, 3-ADON, 15-ADON, ZEN, HT-2, T-2, FB1, FB2, ENA, ENA1, ENB and ENB1 were evaluated. A total of 7 mycotoxins (DON, ZEN, FB1, FB2, ENA1, ENB, and ENB1) out of 12 Fusarium toxins were detected in samples. DON was found in infant cereal, infant cracker, infant noodle, wheat flour, rice, and millet samples. Detection rates for DON in cereal-based infant foods ranged from 1.5 to 91.7%, with the highest detection rate in infant noodle samples (Table 3). Acetylated compounds of 3-ADON and 15-ADON were not detected in samples analyzed in this study, which was consistent with the study by Vin et al. (2020). In Table 3, the mean value of DON was 26.6 $\mu\text{g}/\text{kg}$ in cereal-based infant foods; the average value of DON was 78.8, 48.1, 22.4, 4.1, 1.8, and 1.1 μ g/kg in wheat flour, infant noodles, infant crackers, rice, millet, and infant cereal, respectively. The highest level of DON with contamination level of 912.3 µg/kg was found in an infant noodle sample, which was within the MRL ($<1000 \ \mu g/kg$) set by China and US but exceeded the MRL set by EU ($<750 \ \mu g/kg$) (Shang & Yang, 2019). In cereal-based infant foods, detection rates for DON varied with the food types, with higher detection rates observed in wheat-based infant foods than those of rice-based infant foods. In previous studies, DON has been detected in infant foods worldwide. Compared to the current study, the incidence of DON in cereal supplements for infants and children was 60.3% (217/360) with the average concentration of 116.3 µg/kg and the highest concentration of 1198.7 µg/kg (Wang et al., 2019). DON was detected in 76.0% of commercial baby foods with a higher average concentration of 102.6 µg/kg (Juan et al., 2014). DON was detected in breakfast cereals in the range of 96-210 µg/kg, in infant-specific foods in the range of 112-133 µg/kg (Zhang, Flannery, Oles, & Adeuya, 2018), and in cereal-based baby foods up to 286 µg/kg (Cano-Sancho, Gauchi, Sanchis, Marín, & Ramos, 2011). DON was found in 75% of wheat-based teething crackers with the mean of 45 μ g/kg. Rice-based cereal samples were contaminated with the least number of mycotoxins, which was consistent with our study (Lombaert et al., 2014).

ZEN was detected in infant cereal, cracker, noodle, and millet samples except of wheat flour and rice samples. Detection rates for ZEN in cereal-based infant foods were in the range of 0-66.7% depending on the food types, with the highest detection rate in millet samples. The average value of ZEN was 0.2 $\mu g/kg$ in cereal-based infant foods. The highest concentration of ZEN (8.8 µg/kg) was observed in a millet sample (Table 3). Overall, no samples with ZEN contamination levels exceeded the MRL set by China (<60 μ g/kg) and EU (<100 μ g/kg) (Shang & Yang, 2019), which were in line with previous studies (De Boevre et al., 2013; Vin et al., 2020; Škrbić, Antić, & Cvejanov, 2017). It was reported that the occurrence levels of ZEN ranged from 0.19 to 17 µg/kg in cereal products (De Boevre et al., 2013; Yau et al., 2016), 2.64 μ g/kg in a cracker sample (Škrbić et al., 2017), and 12 μ g/kg in crackers (Warth et al., 2012). In contrast, Oueslati, Berrada, Manes, and Juan (2018) reported that relative high level of ZEN was found in baby mix (44 μ g/kg), bsissa (41 μ g/kg), and semolina (23 μ g/kg). The occurrence levels of ZEN were in the range of 27–905 $\mu g/kg$ in bread samples (Saladino et al., 2017). These above results indicated that ZEN contamination may not be a serious problem in cereal-based infant foods

Detection rates for FB1and FB2 in cereal-based infant foods were in the range of 0–9.3% and 0–1.1%, respectively, depending on the food types (Table 3). FB1 and FB2 was detected in infant cereal, cracker, noodle, millet, and wheat flour except of rice samples. Contamination levels of FB1 and FB2 in positive samples ranged from 1.2 to 252.4 μ g/ kg, with the highest concentration of FB1 (252.4 μ g/kg) found in an infant noodle sample (Table 3). The average contamination level of FB1 and FB2 was 2.2 μ g/kg and 0.1 μ g/kg in cereal-based infant foods. In this study, contamination of FB1 and FB2 were mainly present in infant noodles and infant crackers, which may be attributed to the variety of food ingredients (e.g., corn starch and corn oil) added in the recipes of infant noodles and crackers. In China, there is no MRL set for FB1 and FB2 (GB 2761–2017). The MRL of the sum of FB1 and FB2 in infant foods is 200 μ g/kg set by the EU (Yang et al., 2020). Two infant noodle samples were contaminated with 242.3 μ g/kg and 252.4 μ g/kg of FB1, which exceeded the standards of 200 μ g/kg set by EU. The contamination of FB1 and FB2 in cereal-based infant food has been reported in previous studies. Vin et al. (2020) reported that detection rates of FB1 and FB2 were in the range of 7% and 18% in infant foods, which was significantly higher than those of found in this study. Another study reported that the total of FB1 and FB2 was 36.4 μ g/kg in infant-specific food and 119 μ g/kg in corn snacks, respectively (Cano-Sancho et al., 2011).

In this study, ENs (ENA, ENA1, ENB, and ENB1) were found in infant cracker, infant noodle, wheat flour, and rice samples, but no ENs were detected in infant cereal and millet samples. Detection rates of ENs were in the range of 0-41.2% depending on the food types. The mean level of ENs ranged from 0.2 to 4.7 μ g/kg depending on the sample types; the highest level of 33.9 µg/kg for ENB1 was detected in a rice sample (Table 3). Previous studies have reported the occurrence of ENs in cereal-based foods. Braun et al. (2021) found low levels of ENs (<40 $\mu g/kg$) with the highest incidence rate up to 60% of processed cereal-based infant foods. The detection rates of ENB, ENB1, ENA, and ENA1 found in cereal-based infant foods in Italy market was 70%, 26%, 13%, and 9%, respectively (Juan et al., 2014). The occurrence levels of ENs were in the range of 11.8–832 μ g/kg and the maximum contamination of ENB was 832 µg/kg detected in a cereal-based baby food sample (Juan et al., 2014). Serrano, Meca, Font, and Ferrer (2012) reported that detection rates of ENA, ENA1, ENB and ENB1 were 2.2%, 13.3%, 2.2%, and 40% of cereal-based baby foods with the maxim concentration levels of 149.6, 101.7, 39.4 and 35.8 mg/kg, respectively. Although the incidences of ENs in food samples are mostly lower than those of the legislated mycotoxins, such as AFB1 or OTA, their presence in cereal-based infant foods should increase the concern about their possible impacts on human health, especially on infants.

3.2.2. Alternaria toxins

Cereal grains are commonly infected by fungus of Alternaria, and the incidence of Alternaria toxins detected in grains was exceedingly high. Alternaria species are capable of producing a number of mycotoxins. Among them, AME, AOH, and TeA are the most frequent Alternaria toxins found in foods (Scott, Zhao, Feng, & Lau, 2012). In this study, AME, AOH, and TeA were found in infant cracker, infant noodle, wheat flour, rice, and millet samples except of rice and infant cereal samples. The incidence rate of AME was 7.9%, 6.2%, 4.8%, and 4.3% in infant crackers, wheat flour, infant noodles, and millet samples at levels below 7.1, 8.8, 3.1, and 3.2 µg/kg, respectively (Table 3). The average contamination level AME was 0.1 µg/kg in cereal-based infant foods analyzed in this study. Previous studies have reported low levels of AME detected in cereal and its derived products. Braun et al. (2021) reported that AME was found in cereal-related samples, with the highest concentration up to 1.1 μ g/kg detected in a commercial oatmeal sample. The incidence rate of AME was 90% in infant foods with the highest contamination level of 9.0 $\mu g/kg$ found in a multigrain cereal sample (Scott et al., 2012). In this study, AOH was detected in 16.7%, 15.9% 7.9%, 4.3%, and 3.2% of wheat flour, millet, infant noodles, and infant cracker samples at levels below 45.2, 3.5, 3.2, and 3.1 µg/kg, respectively (Table 3). No AOH was detected in infant cereal and rice samples. The average contamination level AOH was 0.3 $\mu g/kg$ in cereal-based infant foods, and the mean level of AOH ranged from 0.2 to 3.3 μ g/kg depending on sample types. High incidence of AOH and high contamination levels of AOH in cereal-related samples were reported in previous published works. Scott et al. (2012) reported that the detection rate of AOH was 90% in infant foods with the highest concentration up to 4.4 µg/kg. The highest concentration of AOH and AME detected in cereals and cereal-based products was 256 and 86 µg/kg, respectively (European Food Safety Authority EFSA, 2011), which were significantly

The results of mycotoxins detected in processed infant foods (A) and common cereals (B) marketed in China.

A) Processe	d infant foods															
Analytes	Infant cereal (n = 133)				Infant cracker ((n = 277)				Infant noodle (Infant noodle ($n = 229$)				
	Positive (%)	Content	Content (µg/kg)				Content	Content (µg/kg)				Content	Content (µg/kg)			
		Mean	75th	97.5th	Maximum		Mean	75th	97.5th	Maximum		Mean	75th	97.5th	Maximum	
AFB1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
AFB2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
AFG1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
AFG2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
OTA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8(3.5)	0.2	0.2	1.8	3.0	
DON	2(1.5)	1.1	0.5	0.5	65.5	171(61.7)	22.4	34.7	99.8	282.2	210(91.7)	48.1	57.2	200.5	912.3	
3-ADON	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
15-ADON	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
ZEN	3(2.3)	0.2	0.2	1.9	2.3	9(3.2)	0.2	0.2	1.4	6.4	6(2.6)	0.2	0.2	1.0	2.9	
HT-2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
T-2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
FB1	2(1.5)	0.2	0.2	0.2	1.2	12(4.3)	1.4	0.2	8.8	35.1	9(3.9)	6.0	0.2	158	252.4	
FB2	n.d.	n.d.	n.d.	n.d.	n.d.	3(1.1)	0.3	0.2	0.2	21.9	2(0.9)	0.3	0.2	0.2	26.0	
ENA	n.d.	n.d.	n.d.	n.d.	n.d.	114(41.2)	2.2	2.5	17.5	32.8	n.d.	n.d.	n.d.	n.d.	n.d.	
ENA1	n.d.	n.d.	n.d.	n.d.	n.d.	28(10.1)	0.4	0.2	2.9	32.8	4(1.7)	0.2	0.2	0.2	2.2	
ENB	n.d.	n.d.	n.d.	n.d.	n.d.	15(5.4)	0.2	0.2	1.7	2.9	22(9.6)	0.5	0.2	3.7	24.8	
ENB1	n.d.	n.d.	n.d.	n.d.	n.d.	11(4)	0.2	0.2	1.5	2.9	21(9.2)	0.6	0.2	3.3	24.0	
AME	n.d.	n.d.	n.d.	n.d.	n.d.	22(7.9)	0.3	0.2	1.4	7.1	11(4.8)	0.2	0.2	1.3	3.1	
AOH	n.d.	n.d.	n.d.	n.d.	n.d.	9(3.2)	0.2	0.2	1.4	3.1	18(7.9)	0.3	0.2	1.8	3.2	
TeA	n.d.	n.d.	n.d.	n.d.	n.d.	115(41.5)	14.4	22.1	79.5	166.4	198(86.5)	26.7	34.6	107.1	164.2	

B) Common cereals

Analytes	Wheat flour $(n = 54)$					Rice (n = 58)					Millet (n = 69)				
	Positive (%)	Content (µg/kg)			Positive (%)	Content (µg/kg)			Positive (%)	Content (µg/kg)		
		Mean	75th	97.5th	Maximum		Mean	75th	97.5th	Maximum		Mean	75th	97.5th	Maximum
AFB1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AFB2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AFG1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AFG2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
OTA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DON	49(90.7)	78.8	85.1	557.8	622.4	14(24.1)	4.1	1.3	27.2	28.6	11(15.9)	1.8	0.5	19.2	23.2
3-ADON	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
15-ADON	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ZEN	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	46 (66.7)	1.7	2.0	8.0	8.8
HT-2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
T-2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FB1	5(9.3)	2.1	0.2	46.5	47.2	n.d.	n.d.	n.d.	n.d.	n.d.	3(4.3)	0.9	0.2	5.8	21.6
FB2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ENA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ENA1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ENB	2(3.7)	0.6	0.2	14.7	22.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ENB1	2(3.7)	0.3	0.2	6.3	9.5	20 (34)	4.7	7.8	33.1	33.9	n.d.	n.d.	n.d.	n.d.	n.d.
AME	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3(4.3)	0.2	0.2	0.6	3.2
AOH	9(16.7)	3.3	0.2	39.0	45.2	n.d.	n.d.	n.d.	n.d.	n.d.	11(15.9)	0.4	0.2	1.4	3.5
TeA	2(3.7)	0.8	0.5	7.9	8.3	n.d.	n.d.	n.d.	n.d.	n.d.	54(78.3)	73.6	70.8	698.7	788.3

*n.d.: non-detection; non-detection was treated as zero during the calculation of the arithmetic mean, 75th, and 97.5th percentiles of the occurrence levels of mycotoxins detected in infant foods.

higher than that of the AOH and AME detected in this study. A contrast study reported that no AOH was detected in cereal-based infant foods and infant formula (Braun et al., 2021).

TeA was found in infant cracker, infant noodle, wheat flour, millet, but no TeA was found in infant cereal and rice samples. Detection rates were in the range of 0-86.5% in cereal-based infant cereals for TeA. The highest detection rate of TeA was found in infant noodle samples (86.5%). Overall, the incidence rate of TeA was significantly higher than that of AOH and AME in samples analyzed (Table 3). The average contamination level TeA was 18.4 µg/kg in cereal-based infant foods, and the mean level of TeA in different types of samples ranged from 0.8 to 73.6 µg/kg (Table 3). The highest level of 788.3 µg/kg was detected in a millet sample; the mean value of TeA in millet samples was 73.6 µg/kg which was significantly higher than that of AME and AOH (Table 3). Previous studies reported that detection rate of TeA was 31.0% with the concentration levels up to 124 μ g/kg in a millet-based sample (Braun et al., 2021). Sorghum-based infant foods, which were not included in the sample types of this study, were reported to contain higher levels of TeA than other types of cereal-based infant foods (Asam & Rychlik, 2013). Currently, there is no regulations set for Alternaria toxins due to their low acute toxicity and no serious direct economic losses caused by their genus. However, due to their universality of infection such as in invading textiles, wallpapers, soil, leaves, fruits, vegetables, and crops (Asam, Konitzer, & Rychlik, 2011), Alternaria toxins are receiving much attention in the last decade.

3.2.3. Aflatoxins occurrence

In this study, no aflatoxins were detected in analyzed samples. A few previous studies reported that aflatoxins were found in infant cereals. Hernandez-Martinez and Navarro-Blasco (2010) reported the occurrence levels of AFB1 ranged from 0.12 to 3.11 $\mu g/kg$ in seven infant cereals in Spain. In Turkey, Baydar, Erkekoglu, Sipahi, and Sahin (2007) reported that detection rates of AFB1 was 87%, and the occurrence level of AFB1 was in the range of 0.10–6.04 μ g/kg with the mean concentration value of $0.89 \,\mu\text{g/kg}$ in infant cereals. Tam et al. (2006) found that the levels of contamination for AFB1, AFB2, AFG1, AFG2 were in the range of 0.002-1.00 µg/kg, 0.002-0.14 µg/kg, 0.008 to 0.27 µg/kg, $0.008-0.048 \ \mu g/kg$, respectively, in breakfast and infant cereal samples in Canada. On the other hand, aflatoxins were not detected in cereal products in Tunisian (Oueslati et al., 2018). The possible reasons for the variations in the detection rates for aflatoxins could be attributed to differences in sample types analyzed, number of samples collected or seasons for sampling.

3.2.4. Ochratoxin A occurrence

Generally, cereal-based foods are inclined to be contaminated with OTA. In this study, the detection rate of OTA was 1.1% of 820 samples evaluated, which was significantly lower than that of 38% of breakfast cereals and 17% of cereal-based baby foods (Kabak, 2009). In this study, OTA was only detected in infant noodle samples. The average contamination level OTA was 0.02 µg/kg in cereal-based infant foods. In infant noodle samples, the detection rate for OTA was 3.5%, the average contamination level of OTA 0.2 μ g/kg, and the highest contamination level of $3.0 \,\mu\text{g/kg}$ was observed which was satisfied with the MRL set by China (<5 µg/kg) and EU (<3 µg/kg) (Shang & Yang, 2019). In previous studies, OTA was detected in different types of infant foods around the world. Warth et al. (2012) reported that OTA contamination was found in commercial infant formulas (max. 3.2 µg/kg). Ozden, Akdeniz, and Alpertunga (2012) found that the incidence of OTA was 21.62%, 19.05%, and 55.95% in breakfast cereals, cereal-based baby foods, and tarhana samples, respectively, with the mean concentration of 0.32, 0.14, and 0.41 µg/kg, respectively. Beltrán et al. (2011) reported that detection rate for OTA was 14% with the highest contamination level up to 0.05 μ g/kg in a cereal infant formula in Spain. Juan et al. (2014) found 0.06 µg/kg of OTA in an infant cereal. Oueslati et al. (2018) detected the occurrence levels of OTA up to 66 μ g/kg in basissa samples,

whereas OTA was not detected in cereal-based baby foods from Morocco (Zinedine et al., 2010). OTA was suspected to be a possible cause of a chronic kidney disease known as "Balkan Endemic Nephropathy" and an increased incidence of tumors of the upper urinary effect (Stefano, 2019). Although the results in previous studies and the current study showed low levels of OTA contamination in cereal-derived products, the potential health effects caused by OTA contamination should not be ignored.

3.3. Co-occurrence of mycotoxins in samples

Since a single species of fungi can produce several metabolites and several species of fungi may produce various toxins simultaneously, agro-products are commonly contaminated with multi-mycotoxins (Ibáñez-Vea, Martínez, González-Peñas, Lizarraga, & López de Cerain, 2011). Overall, mycotoxin co-contamination was evident in 48.5% (398/820) of analyzed samples. The co-occurrence of two mycotoxins accounted for 31.1% of cereal-based infant foods, which was the highest, followed by the co-occurrence of three mycotoxins (12.1%), four mycotoxins (5.2%), five mycotoxins (2.4%), six mycotoxins (0.1%), seven mycotoxins (0.4%), and eight mycotoxins (0.1%). Co-occurrence of mycotoxins varied depending on sample types. Mycotoxin co-occurrence was observed in 86.9%, 62.3%, 57.0%, 29.6%, 6.9%, and 1.5% in infant noodle, millet, infant cracker, wheat flour, rice, and infant cereal samples. Different combinations of co-occurrence of mycotoxins in cereal-based infant foods marketed in China were shown in Table 4. The most common combination of co-occurrence mycotoxins was DON and TeA, which was found in 161 of 820 (19.6%) of all cereal-based infant products. Co-occurrence of DON and ENA accounted for 3.4% of the samples, followed by co-occurrence of three mycotoxins of DON, TeA, and ENA with the occurrence of 3.0% of all samples (Table 4). According to sample types, the predominant combination of co-occurrence mycotoxins was DON and TeA in infant noodles, DON and ENA in infant crackers, ZEN and FB1in infant cereal, DON and ENB1 in rice, ZEN and TeA in millet, and DON and AOH or DON and ZEN in wheat flour samples. Multi-occurrence cases of mycotoxins were reported in previous studies. Oueslati et al. (2018) reported that 32% (37/117) of samples were present with co-occurrence of different mycotoxins, and a combination of two toxins was commonly observed cases. Zhang et al. (2018) reported that co-occurrence of mycotoxins was observed in 12% and 32% of infant foods and breakfast cereals, respectively, and co-occurrence of DON and ZEN was the prevalent combination. Different types of mycotoxins co-occurrence might contribute to the influences of environmental conditions in different areas. Considering possible synergistically harmful effects on human health, co-occurrence of mycotoxins in infant foods should conduct further research.

3.4. Contamination patterns

In this study, low levels of mycotoxins derived from Aspergillus, Fusarium, and Alternaria fungi were detected in cereal-based infant foods marketed in China. Among infant noodles, infant crackers, millet, and wheat flour samples, the predominant contamination pattern of DON + TeA (Table 4) was produced both from Fusarium spp and Alternaria spp. The notable fungi were Alternaria spp. which produce Alternaria toxins (AME, AOH, and TeA), accounted for 33.0%, 17.6%, and 32.9% contamination in infant noodles, infant crackers, and millet samples (Fig. 1). Fusarium toxin (DON, ZEN, and FB1), commonly produced by Fusarium spp., were detected in rice-based infant cereals, accounting for 0.5% (Fig. 1). Generally, rice samples only contained toxins produced from Fusarium spp., which was further proved in this study. Here, only infant noodle and cracker samples contained a small percentage of toxins (OTA) produced by Aspergillus spp. The contamination pattern is usual considering fungus Fusarium commonly infect cereals and produce toxins (Mateo, Mateo, & Jimenez, 2002). A contrast study reported that

Different combinations of co-occurrence of mycotoxins in cereal-based infant foods marketed in China.

N*	Combination	Incidence (total 820)	Frequency (%)	N*	Combination	Incidence (total 820)	Frequency (%)
1	DON	95	11.6	3	AME + TeA + ENB1	1	0.1
	TeA	42	5.1		AME + AOH + TeA	1	0.1
	ENB1	19	2.3		DON + AME + ENB1	1	0.1
	ZEN	10	1.2		FB1 + TeA + ENA	1	0.1
	ENA	6	0.7		DON + ZEN + ENA	1	0.1
	AOH	6	0.7		DON + AOH + ENA	1	0.1
	FB1	2	0.2		ZEN + FB1 + TeA	1	0.1
	OTA	1	0.1		DON + FB1 + AOH	1	0.1
	AME	1	0.1		DON + ZEN + FB1	1	0.1
2	DON + TeA	161	19.6	4	DON + TeA + ENA + ENA1	9	1.1
	DON + ENA	28	3.4		DON + TeA, + ENB + ENB1	6	0.7
	ZEN + TeA	16	2.0		DON + AME + TeA + ENA	3	0.4
	DON + ENB1	10	1.2		DON + AME + AOH + TeA	2	0.2
	TeA + ENA	10	1.2		DON + AOH + TeA + ENB1	2	0.2
	DON + ZEN	8	1.0		DON + FB1 + AOH + TeA	2	0.2
	DON + AME	4	0.5		DON + FB1 + TeA + ENA	2	0.2
	AME + TeA	3	0.4		DON + ZEN + TeA + ENA	2	0.2
	ZEN + FB1	3	0.4		DON + ZEN + FB1 + FB2	2	0.2
	DON + AOH	3	0.4		ZEN + AME + AOH + TeA	2	0.2
	FB1 + FB2	2	0.2		DON + ZEN + AOH + TeA	2	0.2
	DON + FB1	2	0.2		DON + FB1 + FB2 + TeA	1	0.1
	FB1 + TeA	1	0.1		DON + ZEN + FB1 + TeA	1	0.1
	ENA1 + ENB	1	0.1		OTA + DON + FB1 + TeA	1	0.1
	TeA + ENB1	1	0.1		DON + ZEN + AME + TeA	1	0.1
	ZEN + AOH	1	0.1		DON + TeA + ENA + ENB1	1	0.1
	DON + ENB	1	0.1		DON + ENA + ENA1 + ENB1	1	0.1
	AME + AOH	1	0.1		DON + FB1 + AME + ENA	1	0.1
3	DON + TeA + ENA	25	3.0		OTA + DON + TeA + ENA	1	0.1
	DON + TeA + ENB1	14	1.7		ZEN + FB1 + TeA + ENB	1	0.1
	$\mathrm{DON} + \mathrm{ENB} + \mathrm{ENB1}$	9	1.1		DON + FB1 + AOH + TeA	1	0.1
	DON + ZEN + TeA	6	0.7	5	DON + TeA + ENA + ENA1 + ENB	7	0.9
	ZEN + TeA + ENB	5	0.6		DON + TeA + ENA1 + ENB + ENB1	3	0.4
	ZEN + AOH + TeA	5	0.6		DON + AME + AOH + TeA + ENB1	3	0.4
	OTA + DON + TeA	4	0.5		DON + AOH + TeA + ENB + ENB1	2	0.2
	DON + AOH + TeA	4	0.5		OTA + DON + AME + AOH + TeA	1	0.1
	DON + AME + TeA	3	0.4		DON + FB1 + AME + AOH + TeA	1	0.1
	DON + ENA + ENA1	3	0.4		DON + FB1 + ENA + ENA1 + ENB	1	0.1
	ENA + ENA1 + ENB	3	0.4		ZEN + AME + AOH + TeA + ENB	1	0.1
	DON + AME + ENA	3	0.4	6	DON + AOH + TeA + ENA + ENA1 + ENB	1	0.1
	DON + FB1 + ENA	2	0.2	7	OTA + DON + AME + AOH + TeA + ENB + ENB1	1	0.1
	DON + TeA + ENB	1	0.1		$\mathrm{DON} + \mathrm{AOH} + \mathrm{TeA} + \mathrm{ENA} + \mathrm{ENA1} + \mathrm{ENB} + \mathrm{ENB1}$	1	0.1
	DON + FB2 + TeA	1	0.1		$\mathrm{DON} + \mathrm{AME} + \mathrm{AOH} + \mathrm{TeA} + \mathrm{ENA} + \mathrm{ENA1} + \mathrm{ENB}$	1	0.1
	DON + FB1 + TeA	1	0.1	8	$\mathrm{DON} + \mathrm{ZEN} + \mathrm{AME} + \mathrm{AOH} + \mathrm{TeA} + \mathrm{ENA1} + \mathrm{ENB} + \\$	1	0.1
					ENB1		

N* : number of co-occurrence of mycotoxins.



Fig. 1. Contamination patterns of co-occurrence of multiple mycotoxins in cereal-based infant foods.

rice-based samples were mostly contained with toxins from *Alternaria* spp., but no rice-based samples were contaminated with mycotoxins from *Fusarium* spp. (Braun et al., 2021). Difference of contamination

patters of mycotoxins in infant foods might be caused by the differences in the agriculture practice, food production, or the climate conditions.

3.5. Risk assessment

The exposure results for detected mycotoxins to infants are listed in Table 5. In this study, risk assessment to mycotoxins was calculated deterministically based on consumption data of cereal-based infant foods, body weight of Chinese infants, and the occurrence levels of mycotoxins detected. The mean chronic dietary exposure and the 97.5th percentile chronic dietary exposure to mycotoxins through cereal-based infant food consumption in infants are shown in Table 5.

As shown in Table 5, the dietary intakes of DON were 0.11 μ g/kg b. w./day and 0.31 μ g/kg b.w./day for average infant consumers (mean chronic exposure) and the heavy infant consumers (97.5th percentile chronic exposure), respectively. These values are much lower than the TDI of 1.0 μ g/kg b.w./day set by JECFA (EFSA, 2017). The HQs of DON for the average infant consumers (10.96%) and the heavy infant consumers (30.6%) were much less than 1, indicating an acceptable dietary risk of DON for Chinese infants through cereal-based food consumption. Similar results were reported in previous studies. Wang et al. (2019) reported that the dietary exposure of DON was 0.27 μ g/kg b.w./day for average infant consumers. Braun et al. (2021) reported that the dietary

Chronic dietary risk assessment for mycotoxins through consumption of cerealbased infant foods by Chinese infants.

Analytes	TDI ^a / TTC ^b	Mean concentration	EDI (µg/ day)	kg b.w./	HQ (%)		
	(µg/kg b.w./ day)	(µg/kg)	Mean ^d	97.5th ^e	Mean ^d	97.5th ^e	
DON	1.00 ^a	26.05	0.11	0.31	10.96	30.60	
ZEN	0.50 ^a	0.58	0.002	0.01	0.48	1.35	
Sum of HT-2 and T- 2	0.06 ^a	-	-	-	-	-	
Sum of FB1 and FB2	2.00 ^a	1.14	0.005	0.01	0.24	0.67	
OTA	0.10 ^a	0.10	0.0004	0.001	0.42	1.17	
ENs	_	4.38	0.02	0.05			
ENA	-	2.20	0.01	0.03			
ENA1	-	0.30	0.001	0.004			
ENB	-	0.43	0.002	0.005			
ENB1	-	1.45	0.01	0.017			
AME	0.0025 ^b	0.37	0.002	0.004	61.69	172.27	
AOH	0.0025 ^b	1.05	0.004	0.01	176.67	493.31	
TeA	1.50^{b}	28.80	0.12	0.34	8.08	22.55	
AFB1	ALARA ^c	-	-	-	-	-	
AFB2	ALARA	-	-	-	-	-	
AFG1	ALARA	-	-	-	-	-	
AFG2	ALARA	-	-	-	-	-	

Note.

 $EDI = (mean \ concentration \ of \ mycotoxins \ in \ cereal-based \ infant \ foods \times \ consumption \ of \ cereal-based \ infant \ foods)/body \ weight, \ \mu g/kg \ b.w./day. \ During \ EDI \ calculation, \ LOD/2 \ values \ were \ used \ for \ the \ data \ below \ LOD.$

 $HQ = (EDI/TDI \text{ or TTC}) \times 100\%$. Acceptable health risk is considered when HQ is lower than 100%; unacceptable health risk is suspected when HQ is more than 100%.

^a TDI: tolerable daily intake.

^b TTC: threshold of toxicological concern.

^c ALARA: as low as reasonably achievable.

^d Mean daily cereal-based products consumption is 0.046 kg.

^e 97.5th percentile (heavy consumers) cereal-based products daily consumption is 0.129 kg.

exposure of DON was 0.33 μ g/kg b.w./day for infants. Kirimer et al. (2020) reported that the estimated mean chronic dietary exposures to DON and the 95th percentile exposure to DON was 0.161 and 0.564 μ g/kg b.w./day in infants, respectively. EFSA reported that the dietary exposure of DON to young children was between 0.16 μ g/kg b.w./day and 0.73 μ g/kg b.w./day for infants in 2013, and between in 0.6 μ g/kg b.w./day and 2.0 μ g/kg b.w./day for infants in 2017 (EFSA, 2017). Overall, the dietary exposure of DON for infants was no more than the threshold level of 1 μ g/kg b.w. per day, indicating an acceptable health risk.

As shown in Table 5, the estimated mean and 97.5th percentile exposures to ZEN and the sum of FB1 and FB2 ranged from 0.002 to 0.01 μ g/kg b.w./day, respectively. These values are far lower than the provisional tolerable maximum daily intake (PMTDI) of 0.5 μ g/kg b.w./day and 2.0 μ g/kg b.w./day set for ZEN and the sum of FB1, FB2 and FB3 by JECFA (Yang et al., 2020), respectively. The HQs of ZEN and the sum of FB1 and FB2 were lower than 1 in this study, indicating an acceptable health risk for Chinese infants through consumption of cereal-based foods. A similar study conducted by Braun et al. (2021) reported that the dietary exposure for ZEN and FB1 was 0.083 μ g/kg b.w./day and 0.40 μ g/kg b.w./day, respectively, which was far lower than the TDI value set by the JECFA and EFSA (Yang et al., 2020). In 2011, EFSA reported that exposure to ZEN was lower than 0.1 μ g/kg b.w./day for children under 3 years old (European Food Safety Authority EFSA, 2011).

Hexadepsipeptides, like ENs, were frequently detected in cerealbased commodities, but dietary risk assessment could not be conducted for ENs due to lack of its HBGV. Therefore, only dietary intakes were estimated. In this study, as for ENs (ENA, ENA1, ENB, and ENB1), the chronic dietary intake of ENs was estimated to be in the range of 0.002 μ g/kg b.w./day to 0.05 μ g/kg b.w./day for the average and heavy consumers, respectively, in this study (Table 5). Braun et al. (2021) reported that the dietary intakes of ENA, ENA1, ENB, ENB1 were in the range of 0.005–0.5 μ g/kg b.w./day based on the hypothetical upper bound intake, which was slightly higher than the results obtained in this study. To the best of our knowledge, only limited studies have evaluated ENs contamination in infant foods.

As for Alternaria toxins, including AME, AOH, and TeA, the threshold of toxicological concern (TTC) approach was used to estimate the dietary risk of AME, AOH, and TeA for infant health as recommended by the literatures (European Food Safety Authority EFSA, 2010; Kroes et al., 2004). The TTC value of 0.0025 μ g/kg b.w./day was used for both AME and AOH, and the TTC value of 1.5 μ g/kg b.w./day was used for TeA during risk assessment (European Food Safety Authority EFSA, 2010; Kroes et al., 2004). For AME, the chronic mean exposure of 0.002 μ g/kg b.w./day was lower than the value of TTC (0.0025 μ g/kg b.w./day), but the dietary risk of AME for heavy consumer exceeded 60% of TCC set for AME, and the corresponding HQ was 172.27% (>1), indicating a dietary exposure risk for infants (Table 5). In this study, the mean chronic dietary exposure to AOH was 0.004 µg/kg b.w./day, and the 97.5th percentile chronic exposure was 0.01 µg/kg b.w./day, which were much higher than the value of TCC set for AOH (0.0025 µg/kg b.w./day). If so, the relevant HQs were much higher than 1. As for genotoxic AME and AOH, the chronic dietary exposure exceeded the relevant TCC value in this study, which was similar to the results reported by EFSA (European Food Safety Authority EFSA, 2011), indicating a potential dietary risk for Chinese infants, but additional toxicity data were needed for making accurate estimates.

For nongenotoxic TeA, the mean chronic exposure to TeA was 0.12 μ g/kg b.w./day, and the 97.5th percentile exposure to TeA was 0.34 μ g/ kg b.w./day for infant consumers. The values of dietary intakes were much lower than the TCC value (1.5 µg/kg b.w./day) set for TeA by EFSA (European Food Safety Authority EFSA, 2010), and the corresponding HQs were 8.08% and 22.55%, respectively, much lower than 1, indicating low dietary exposure of TeA through cereal-based foods in Chinese infants. The results obtained in this study was consistent with the results reported by EFSA (European Food Safety Authority EFSA, 2011) that TeA was considered unlikely to be a human health concern. This is the first study about AME, AOH, and TeA dietary exposure for infants in China. Another study conducted by Zhao, Shao, Yang, Li, and Zhu (2015) reported similar dietary exposure results of AME, AOH, and TeA for the Chinese general population. The results showed that the dietary exposure of AME and AOH exceeded the relevant TTC, which confirmed the need for further study to assess the potential health risks, especially in heavy intake consumers. Dietary exposure of TeA through wheat-based product intake was lower than relevant TTC, but additional concern for TeA is warranted because of possible synergistic or additive effects of TeA with AOH, AME in food matrices.

It is important to note that there were some uncertainties in assessment of dietary exposure to the mycotoxins detected in this study for Chinese infants. Firstly, toxin data for dietary risk assessment should be obtained not only from cereal-based food types but also from other food types intended for infant consumption. Secondly, food consumption data used in this study was the average consumption data for infants obtained in a survey conducted in 2015 in China, and food types and intakes also change with the development of society. Thirdly, infants may be exposed to various mycotoxins or toxic chemicals with multiple exposure pathways in daily life. Different mycotoxins or chemicals may pose the synergistic or additive effects to infants. To the best of our knowledge, this is the first study to evaluate multi-mycotoxins both in processed infant foods and common cereals for infant consumption. The

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results of this study could be a useful reference for consumers and producers to be aware of the possible health risks related to infant foods.

4. Conclusions

Multi-mycotoxins in cereal-based infant foods marketed in China were analyzed. Low levels of 12 types mycotoxins were found in 73.7% of samples. DON was the most detected type of mycotoxins in different types of cereal-based infant foods. High levels of TeA were found in millet samples. More varieties of mycotoxins were detected in infant crackers, infant noodles and wheat flour than in infant cereal and rice samples. Co-occurrence of mycotoxins detected in infant foods were further supported by this study. Chronic dietary intake risk assessment of mycotoxins for infants were evaluated through cereal-based infant food consumption. The results showed that the dietary risks associated with DON, ZEN, FB1, FB2, OTA and TeA were acceptable for Chinese infants, but the chronic dietary risk estimated for AME and AOH in infants was not acceptable. Mycotoxins cannot be eliminated completely from the grain supply and are difficult to destroyed during processing. In order to safeguard infant food safety, effort to control mycotoxin contamination in food commodities should involve the whole industrial chain from agriculture practices to the table. Periodic monitoring of infant foods and their ingredients for the occurrence of mycotoxins is an important way to safeguard infant food safety.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Xiaofeng Ji: conceived, designed, and planned the experiments, Formal analysis, UPLC-MS/MS measurement, data evaluation, interpretation and drafted the paper. Yingping Xiao: Formal analysis, participated in sample collection and data analysis. Wen Wang: Formal analysis, participated in sample collection and data analysis. Wentao Lyu: Formal analysis, participated in sample collection and data analysis. Xiaoli Wang: Formal analysis, participated in sample collection and data analysis. Yan Li: Formal analysis, participated in sample collection and data analysis. Tao Deng: Formal analysis, was involved in sample analysis and data analysis. Hua Yang: Supervision, designed and supervised the study and data evaluation/interpretation, All authors contributed to manuscript writing.

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