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Co-occurrence and exposure assessment of deoxynivalenol and fumonisins from maize and maize-based products in Indonesia

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Abstract

This study was conducted to investigate the co-occurrence of deoxynivalenol (DON) and fumonisins (FBs) in maize-based food products and their dietary exposure to the Indonesian population. A survey of 45 samples of maize and maize-based-food products collected from markets in Indonesia were analyzed using liquid chromatography-mass spectrometry after a Quick Easy Cheap Effective Rugged Safe (QuEChERS) extraction and clean-up process. Results revealed that 35.56% of maize food products were found to contain DON and FBs simultaneously. FBs frequently detected in 84.44% of samples and DON was found in 42.22% of the samples with mean positive levels ranging from 0.12 to 264.24 µg/kg (FBs) and 2.62 µg/kg to 122.28 µg/kg (DON). None of the quantifiable samples exceeded the maximum limits established by the European Commission (EC) and National regulation. The highest total means exposure to DON and FBs was found in infants and toddler attaining up to 0.025 and 0.087 µg/kg body weight (bw) per day while 95th percentile (P95) values were 3.8 and 4.4 times higher than the mean exposure. Both for DON and FBs, total mean and P95 exposures of the population did not exceed the provisional maximum tolerable daily intake (PMTDI) of 1 µg/kg bw/day for DON and 2 µg/kg bw/day for FBs, respectively. Therefore, the risk assessment indicates that there is no potential risk to health from DON and FBs through maize and maize-based food intake.

1. Introduction

Mycotoxin exposure to humans is a public health problem worldwide. The Food and Agriculture Organization of the United Nations (2005) estimates that a 40% decrease in life expectancy in developing countries is related to mycotoxin presence in daily food consumption (Martins *et al*., 2012). The most reported fusarium toxins are trichothecenes, zearalenone, and fumonisins, which could be found single or simultaneously in various foods, including maize and its processed products (Hove *et al*., 2016; Jiang *et al*., 2019). Among these mycotoxins, Deoxynivalenol (DON) and Fumonisins (FBs) are the most common mycotoxins found in maize and its processed products (Pleadin *et al*., 2012; Escobar *et al*., 2013; Hove *et al*., 2016; Jiang *et al*., 2019).

DON belongs to the B-trichothecenes group, which has a cyclic sesquiterpenoid structure; produced predominantly by the *Fusarium graminearum* and *Fusarium culmorum*. Chronic effects caused by exposure to DON in humans are still very limited; however, in animals, it can cause weight loss, anorexia, and vomiting effects. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) set a provisional maximum tolerable daily intake (PMTDI) value for DON exposure at 1 µg/kg body weight (bw)/day (JECFA, 2011). Fumonisins (FBs) are produced by *Fusarium verticillioides* and *Fusarium proliferatum*, which commonly contaminate maize (Cano-Sancho *et al*., 2012). Fumonisin B1 (FB1) and Fumonisin B2 (FB2) are the main toxins that occur in the agricultural environment (Kirimker *et al*., 2020), and FB1 is the most dominant fumonisins toxin in maize (70 - 80% of the total FBs is FB1). Moreover, FB1 is the most studied due to considerations of its toxicity (Fodor *et al*., 2015; JECFA, 2017). FB1 is classified as group 2B (possibly carcinogenic to humans) by The International Agency for Research on Cancer (IARC), JECFA (2017) set a PMTDI of 2 µg/kg bw/day for FB1 alone or in combination (FB1 and FB2).

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In Indonesia, maize is one of the main food commodities and is used not only for animal feed but for many other purposes. Maize has industrial uses and serves as a staple food in some regions like East Java where "sego jagung" and "nasi empok" (dishes using maize kernels) were made, and East Nusa Tenggara; while in some regions it is used to prepare traditional food such as "binte bilihuta" (a salty porridge with fish) which comes from Gorontalo.

In order to protect public health, the Codex Alimentarius Commission (CAC, 2019) has set a maximum standard and the European Commission (EC, 2007) has established a maximum level of DON and FBs which can be present in maize and their processed products based on the scientific opinions and the assessment of dietary intakes. The occurrence and contamination level data of DON and FBs from agricultural products such as maize and their products was very limited, especially in Indonesia. In Indonesia, the use of maize for food reached 4.7 million tonnes in 2018 (Ministry of Agriculture, 2019). A survey to evaluate the contamination levels of Fusarium toxins such as DON and FBs in maize based-food for human consumption in Indonesia was reported by several authors (Ali *et al*., 1998; Nuryono *et al*., 2004; Setyabudi *et al*., 2012), however the dietary assessment was mainly conducted on the cancer-causing mycotoxin; Aflatoxin B1 (Ambarwati e*t al*., 2011; Nugraha *et al*., 2018; Rahayu *et al*., 2020). Co-occurrence and dietary exposure assessment of DON and FBs from maize and maize-based food products in Indonesia are reported here.

2. Materials and methods

2.1 Samples collection

During the period of September-December 2020, a total of 45 maize and maize-based food products were randomly collected from retail stores and supermarkets in the cities of Bogor, Depok, and Bekasi, Indonesia. The samples of maize-based food products consisted of fresh maize (n = 5), maize kernels (n = 3), maize flours (n = 6), corn starch (n = 6), rice corn (n = 4), cornflakes (n = 3), corn vermicelli (n = 4), and maize-based snacks (n = 13). Each sample was collected in a quantity of a least 1 kg, placed in an air-tight plastic bag, encoded, and stored at room temperature (25°C) before being transported to the National Accredited Private Laboratory of Food Control. In order to obtain a homogeneous sample, each 250 g of the collected sample was blended and ground using *Waring* blender and laboratory grinder.

2.2 Sample preparation

DON and FBs were extracted from samples with a previously validated method based on the Waters Application Document (2015), Shimadzu Document (2014), and Jettanajit and Nhujak (2016). Briefly, 2 g of homogeneous sample was extracted with 10 mL of double distillate waters and a mixture of 10 mL acetonitrile and formic acid solution (95:5 v/v) using a horizontal mechanical shaker with a speed of 2490×*g*. Furthermore, CEN salt (containing 1 g of sodium citrate dihydrate, 0.5 g of sodium hydrogen citrate sesquihydrate, 1 g of sodium chloride, and 4 g of magnesium sulfate) was added to the sample solution and was then shaken for 5 mins before being centrifuged at 2490×*g* at a controlled temperature (4°C) for 15 mins. One mL of supernatant was transferred into a 2 mL tube containing dispersive solid-phase extraction (dSPE, containing 150 mg of magnesium sulfate, 25 mg of primary and secondary amine sorbent, and 25 mg of C18). The sample mixture was then vortexed for 1 min. A centrifugation process was carried out at a speed of 15339×*g* for 3 mins. The supernatant was filtered through a 0.22 µm membrane and transferred into an amber vial before the injection process. All samples were analyzed in duplicate

2.3 LC-MS/MS analysis

DON and FBs were analyzed with a reversed-phase system using ultra-high-performance liquid chromatography (UPLC), Nexera X2, (Shimadzu, Kyoto, Japan) and coupled with a triple quadrupole mass spectrometer 8060 detector (Shimadzu, Kyoto, Japan) with an electrospray ionization (ESI) source. Acquity BEH C18 analytical column (1.7 µm particle size, 2.1×50 mm, Waters) was used for chromatographic separation and equipped with a C18 guard column (1.7 µm particle size, 2.1×5 mm). Mobile phase A; 0.1 % formic acid in water, and mobile phase B; 0.1% formic acid in acetonitrile were used with a time programmed gradient at a flow rate of 0.4 mL/min with an injection volume of 5 µL. The total run time for the analysis was 15 mins. Both DON and FBs were quantified and confirmed by two transitions in the multiple reaction monitoring mode presented in Table 1.

2.4 Method verification

Method verification was carried out on performance indicators including precision, accuracy, LOD and LOQ (limit of quantification). The LOD and LOQ were determined using a signal-to-noise approach as 3 and 10 times greater than the baseline noise of blank matrices spiked with DON and FBs low levels ranging from 0.03 - 10 µg/kg. Precision (repeatability, n = 7) was carried out by comparing a calculated relative standard deviation (RSDr) of replicate measurement on the same day and comparing it to its Coefficient Variance Horwitz (CV

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Table 1. Multiple reaction monitoring and retention time of DON and FBs.

Mycotoxins	Interface	Retention time (min)	Precursor Ion (m/z)	Product ion (m/z)	Collision energy (v)
DON	ESI (+)	0.72	297.05	249.05	- 10
DON	ESI (+)	0.72	297.05	231.1	- 11
FB1	ESI (+)	7.33	722.3	352.3	- 35
FB1	ESI (+)	7.33	722.3	334.2	- 43
FB2	ESI (+)	8.08	706.3	336.25	- 39
FB2	ESI (+)	8.08	706.3	318.25	- 36

Horwitz). The accuracy (n = 7) was determined by spiking at a range of 50 - 500 µg/kg, both DON and FBs respectively, into the sample matrices. The sample matrices consisted of fresh corn, maize kernels, maize flour and corn starch, corn rice and maize-based food snacks were selected to verify the method analysis, respectively.

2.5 Dietary exposure and consumption data

The exposure of DON and FBs from maize and maize-based food products in Indonesia was estimated and compared with its PMTDI of 1.0 µg/kg bw/day (for DON) and 2.0 µg/kg bw/day (for FBs). The dietary exposure to DON and FBs was estimated using point-of-estimate modeling (deterministic approach) with the mean and 95th percentile (P95). The exposure was calculated by multiplying the mean contamination level of DON and FBs in maize and maize-based food products and their consumption rate. Since DON and FBs are chemical contaminants that are likely to be present in food, the mean food concentration was calculated using a lower bound (LB) and upper bound (UB) approach. The LB results were input as a zero value to those samples in which DON and FBs were not detected. The UB was obtained by assigning a value of the limit of detection (LOD) of both DON and FBs respectively to all samples with no detected result (FAO/WHO, 2009).

The consumption data of maize and maize-based food products of the Indonesian population and the bodyweight of respondents data were provided from the Individual Food Consumption Survey (IFCS) conducted in 2014, and the quantities of food consumed by individuals and itemized data were obtained through the 24-hour dietary recall method (Ministry of Health, 2014; Setyowati *et al*., 2018). Maize and maize-based food products consumed were grouped into food groups according to national food category regulations (Indonesian FDA, 2019). Consumption data was obtained for individuals (g/days) and grouped for infants and toddlers (0 - 59 months), children (5 - 12 years), teens (13 - 18 years), adults (19 - 55 years), and elders (>55 years).

2.6 Statistical analysis

The statistical package used was IBM SPSS Statistics version 25.0 of 2017 for Windows (IBM Corp, New York, USA). The level of confidence required for significance was set at $p < 0.01$.

3. Results

3.1 Verified methods of deoxynivalenol and fumonisin

All method verification results for matrices analysis are shown in Table 2. The LOD of maize and maize-based food products ranged from 2.54 - 3.26 µg/kg for DON and 0.03 - 0.17 µg/kg for FBs. LOQs obtained for DON were in the range 8.46 - 10.87 µg/kg and for FBs they varied between 0.11 to 0.59 µg/kg. Precision as RSDr value (repeatability) obtained for DON and FBs were in the range of 1.76 - 7.05% and 4.41 - 12.70%, respectively, with a requirement value is less than ⅔ CV Horwitz ranged from 6.03 to 12.26% for DON and 10.31 to 31.37% for FBs. Accuracy with recovery was in the range of 61.98 to 106.11% for DON and 61.18 to 118.66% for FBs.

3.2 Frequency and concentration of deoxynivalenol and fumonisin in maize and maize-based food products

A summary of frequency and concentration for both DON and FBs in maize and maize-based food products is shown in Table 3. DON was reported in 19 samples, one of which was found in maize kernels (25%, 11.83 µg/kg), two of which were found in maize flour and corn starch respectively (33.33%, 7.89±5.03 µg/kg; 95.96±51.03 µg/kg;), one of which was found in corn vermicelli (25%, 33.28 µg/kg), and seven of which were found in maize-based snack products (70%, 58.37±32.31 µg/kg). Both cereal cornflakes and popcorn contained DON in all samples (n = 3, respectively). In contrast, DON was not reported in any fresh maize samples (n = 5) and corn rice (n = 4). The highest level of DON was found in popcorn samples with mean levels of 122.28±82.32 µg/kg followed by cornflakes with mean levels of 84.88±60.24 µg/kg.

FBs were the most abundant in the sample, with contamination frequency attained to 84.88% (38 samples), of which FB1 and FB2 were detected in all FBs' positive samples. The highest contamination of FBs was found in maize flour (100%, n = 6), with a mean positive sample of 264.24±122.42 µg/kg. Furthermore,

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Table 2. Analytical performance of DON and FBs analysis in different sample matrices.

Sample matrices	N	Precision		Accuracy	LOD	LOQ
DON						
		RSDr	2/3 CV Horwitz	% Recovery	µg/kg	µg/kg
Fresh corn	7	7.05	12.26	69.15 - 85	2.6217	8.7392
Maize kernel	7	6.58	11.92	90.47 - 106.11	2.5365	8.455
Maize flour	7	5.55	6.03	82.16 - 93.87	3.2659	10.8663
Corn starch	7	5.35	12.1	79.88 - 93.87	2.8257	9.4096
Corn rice	7	1.76	7.53	101.69 - 105.05	3.1386	10.462
Maized - based snack	7	2.19	6.7	61.98 - 80.07	3.1019	10.3398
FB1						
		RSDr	2/3 CV Horwitz	% Recovery	µg/kg	µg/kg
Fresh corn	7	10.51	29.87	87.63 - 118.42	0.1187	0.3958
Maize kernel	7	6.31	11.61	72.90 - 100.52	0.1175	0.3918
Maize flour	7	4.67	10.91	85.03 - 97.33	0.1777	0.5924
Corn starch	7	10.01	30,01	87.52 - 118.05	0.103	0.3431
Corn rice	7	7.26	11.76	89.89 - 110.75	0.1118	0.3727
Maized - based snack	7	10.26	26.52	75.85 - 113.80	0.1273	0.4238
FB2						
		RSDr	2/3 CV Horwitz	% Recovery	µg/kg	µg/kg
Fresh corn	7	12.7	31.37	83.08 - 118.52	0.0424	0.1413
Maize kernel	7	8.01	12.33	67.16 - 87.24	0.0654	0.2179
Maize flour	7	4.41	11.58	62.37 - 77.89	0.1058	0.3526
Corn starch	7	10.27	31.34	90.52 - 1 18.66	0.0391	0.1302
Corn rice	7	7.24	12.4	61.18 - 80.46	0.0526	0.1753
Maized - based snack	7	11.21	28.33	78.87 - 118.60	0.0337	0.1121

the contamination level of FBs in maize flour was followed by maize kernels and corn rice with mean positive samples of 147.38±169.15 µg/kg (100%, n = 4) and 111.06±63.21 µg/kg, respectively (100%, n = 4). Similar to both maize kernels and corn rice, maize-based snack products were detected at a 100 % incident rate with a lower contamination level of FBs (80.56±49.32 µg/kg). Both cornflakes and corn vermicelli were also detected at 100% incident rate (n = 3, n = 4) with lower contamination levels (6.15±2.57 µg/kg, 1.65±0.83 µg/kg). Popcorn was found in a lower incident rate (66.67%, n = 3) with a mean positive of samples of 20.80±25.19 µg/kg. FBs were not detected in seven of the samples (100% in fresh maize, n = 5; 16.67% in corn starch, n = 6; 33.33% in popcorn, n = 3). The contamination level of FBs in corn starch samples was found in a small concentration with a mean positive of samples of 3.50±3.28 µg/kg. This value is similar to the corn vermicelli samples, in which FBs were detected in all of its samples (n = 4, 1.65±0.83 µg/kg). The co-occurrences of DON and FBs were reported in 35.56% samples (n = 45), of which nine samples were found in maize-based snack food. Forty of 45 samples were contaminated with at least only DON or FBs. The contamination of DON in this study showed significant differences in contamination levels among 8 different groups of maize and maize-based products (p = 0.004<0.01 for Kruskal-Wallis Test), while a significant difference was also found in contamination levels of FBs among the groups (p = 0.000<0.01 for Kruskal-Wallis Test).

3.3 The estimated daily intake according to two different scenarios and risk characterization of deoxynivalenol and fumonisin

Table 4 presents the dietary exposure to both DON and FBs from maize and maize-based product consumption for five different age groups with a deterministic approach according to two different scenarios: LB and UB, both mean and P95 exposures. The exposure was calculated by multiplying the mean contamination level of DON and FBs in maize and maize-based food products and their consumption rate. According to the individual food consumption survey (IFCS) in 2014, the consumption levels of maize and maize-based food products were 20.69 g/day for infants and toddlers with an average body weight of 12.88 kg, 29.91 g/day for children with an average body weight of 26.71 kg, 34.28 g/day for teens with an average body weight of 45.18 kg, 32.83 g/day for adult with average body weight of 57.55 kg and 32.44 g/day for elders with

Table 3. Frequency and concentration of DON and FBs in maize and maize-based food products.

Sample matrices	N	Frequency (positive/total)			DON				FBs			
		DON	FB1	FB2	Mean±SD (µg/kg)	Range (µg/kg)	Mean LB (µg/kg)	Mean UB (µg/kg)	Mean±SD (µg/kg)	Range (µg/kg)	Mean LB (µg/kg)	Mean UB (µg/kg)
Fresh corn	5	0/5	0/5	0/5	nd	nd	0	2.62	nd	nd	0	0.12
Maize kernel	4	1/4	4/4	4/4	11.83	nd - 11.83	2.96	4.86	147.38±169.15	11.83 - 386.66	147.38	147.38
Maize flour	6	2/6	6/6	6/6	7.89±5.03	4.33 - 11.45	2.63	4.81	264.24±122.42	120.80 - 400.99	264.24	264.24
Corn starch	6	2/6	5/6	5/6	95.96±51.03	59.88 - 132.05	31.99	40.08	4.20±3.28	1.14 - 8.20	3.50	3.52
Corn rice	4	0/4	4/4	4/4	nd	nd	0	3.14	111.06±63.21	19.34 - 163.46	111.06	111.06
Cornflakes	3	3/3	3/3	3/3	84.88±60.24	32.45 - 150.68	84.88	84.88	6.15±2.57	3.33 - 8.34	6.15	6.15
Corn vermicelli	4	1/4	4/4	4/4	33.28	nd - 33.28	8.32	10.44	1.65±0.83	0.72 - 2.45	1.65	1.65
Maize-based snack	10	7/10	10/10	10/10	58.37±32.31	33.87 - 121.60	40.86	43.74	80.56±49.32	28.65 - 197.34	80.56	80.56
Popcorn	3	3/3	2/3	2/3	122.28±82.32	67.05 - 216.90	122.28	122.28	13.87±25.19	2.99 - 38.61	13.87	13.91
Total	45	19/45	38/45	38/45	46.05	nd - 216.90	32.66	35.20	69.90	nd - 400.99	69.82	69.84

nd: not detected (< LOD), Kruskal - Wallis test for contamination level: $p<0.01$.

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Table 4. The mean and 95th percentile (P95) exposure to DON and FBs (µg/kg bw/day) through maize and maize-based food product consumption in different age groups (0 - 59 months, 5 - 12 years, 13 - 18 years, 19 - 55 years, and >55 years).

	Food groups and subgroups	Exposure (µg/kg bw/day)																			
		0 - 59 months				5 - 12 years				13 - 18 years				19 - 55 years				>55 years			
		Mean		P95		Mean		P95		Mean		P95		Mean		P95		Mean		P95	
		LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
DON	**Vegetables and fruits**	0.000	0.002	0.000	0.010	0.000	0.001	0.000	0.006	0.000	0.001	0.000	0.003	0.000	0.001	0.000	0.003	0.000	0.001	0.000	0.003
	Fresh Corn	0.000	0.002	0.000	0.010	0.000	0.001	0.000	0.006	0.000	0.001	0.000	0.003	0.000	0.001	0.000	0.003	0.000	0.001	0.000	0.003
	Cereals and cereal products	0.004	0.006	0.018	0.025	0.002	0.004	0.011	0.017	0.002	0.030	0.008	0.012	0.001	0.002	0.005	0.008	0.001	0.002	0.005	0.009
	Maize kernel	0.001	0.001	0.002	0.003	0.001	0.001	0.005	0.009	0.001	0.001	0.004	0.007	0.000	0.001	0.002	0.004	0.000	0.001	0.003	0.005
	Maize flour	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Corn starch	0.001	0.001	0.005	0.006	0.001	0.001	0.002	0.002	0.000	0.001	0.001	0.002	0.000	0.000	0.001	0.001	0.000	0.000	0.001	0.001
	Corn rice	0.000	0.001	0.000	0.006	0.000	0.001	0.000	0.005	0.000	0.001	0.000	0.005	0.000	0.001	0.000	0.005	0.000	0.001	0.000	0.005
	Cornflakes	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Corn vermicelli	0.001	0.001	0.007	0.009	0.001	0.001	0.004	0.005	0.001	0.001	0.004	0.005	0.000	0.001	0.003	0.003	0.000	0.000	0.002	0.003
	Ready to eat snacks	0.016	0.017	0.080	0.085	0.010	0.011	0.048	0.051	0.003	0.003	0.017	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Maize-based snacks	0.016	0.017	0.078	0.084	0.001	0.008	0.044	0.047	0.002	0.002	0.016	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Popcorn	0.001	0.001	0.000	0.000	0.002	0.002	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Total	0.020	0.025	0.088	0.094	0.013	0.016	0.053	0.056	0.005	0.007	0.022	0.025	0.002	0.003	0.006	0.010	0.001	0.003	0.006	0.010
FBs	**Vegetables and fruits**	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Fresh corn	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Cereals and cereal products	0.056	0.056	0.382	0.382	0.061	0.061	0.393	0.393	0.052	0.052	0.287	0.287	0.038	0.038	0.237	0.237	0.052	0.052	0.278	0.278
	Maize kernel	0.026	0.026	0.094	0.094	0.033	0.033	0.261	0.261	0.027	0.027	0.208	0.208	0.016	0.016	0.119	0.119	0.020	0.020	0.160	0.160
	Maize flour	0.001	0.001	0.000	0.000	0.002	0.002	0.000	0.000	0.001	0.001	0.000	0.000	0.001	0.001	0.000	0.000	0.001	0.001	0.000	0.000
	Corn starch	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Corn rice	0.028	0.028	0.194	0.194	0.025	0.025	0.179	0.179	0.024	0.024	0.181	0.181	0.021	0.021	0.159	0.159	0.030	0.030	0.188	0.188
	Cornflakes	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Corn vermicelli	0.000	0.000	0.001	0.001	0.000	0.000	0.001	0.001	0.000	0.000	0.001	0.001	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000
	Ready to eat snacks	0.031	0.031	0.154	0.154	0.017	0.017	0.087	0.087	0.004	0.004	0.032	0.032	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
	Maize-based snacks	0.031	0.031	0.150	0.150	0.016	0.016	0.086	0.086	0.003	0.003	0.032	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Popcorn	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Total	0.087	0.087	0.386	0.386	0.077	0.077	0.394	0.394	0.057	0.057	0.288	0.288	0.039	0.039	0.238	0.238	0.051	0.051	0.278	0.278

Note: **Bold** letters are food groups.

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Table 5. The mean and high risk (95th percentile) to DON and FBs (%TDI) through maize and maize-based food product consumption in different age groups (0 - 59 months, 5 - 12 years, 13 - 18 years, 19 - 55 years, and >55 years).

	Food groups and subgroups	Risk (%TDI)																			
		0 - 59 months				5 - 12 years				13 - 18 years				19 - 55 years				>55 years			
		Mean		P95		Mean		P95		Mean		P95		Mean		P95		Mean		P95	
		LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
DON	**Vegetables and fruits**	**0.00**	**0.16**	**0.00**	**1.02**	**0.00**	**0.12**	**0.00**	**0.59**	**0.00**	**0.07**	**0.00**	**0.34**	**0.00**	**0.06**	**0.00**	**0.30**	**0.00**	**0.06**	**0.00**	**0.29**
	Fresh Corn	0.00	0.16	0.00	1.02	0.00	0.12	0.00	0.59	0.00	0.07	0.00	0.34	0.00	0.06	0.00	0.30	0.00	0.06	0.00	0.29
	Cereals and cereal products	**0.40**	**0.57**	**1.76**	**2.46**	**0.23**	**0.38**	**1.11**	**1.73**	**0.17**	**0.30**	**0.82**	**1.22**	**0.11**	**0.20**	**0.52**	**0.84**	**0.09**	**0.21**	**0.53**	**0.94**
	Maize kernel	0.05	0.09	0.19	0.31	0.07	0.11	0.52	0.86	0.05	0.09	0.42	0.68	0.03	0.05	0.24	0.39	0.04	0.07	0.32	0.53
	Maize flour	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Corn starch	0.11	0.13	0.47	0.58	0.05	0.07	0.16	0.20	0.04	0.05	0.12	0.15	0.02	0.03	0.10	0.13	0.01	0.02	0.08	0.10
	Corn rice	0.00	0.08	0.00	0.55	0.00	0.09	0.00	0.51	0.00	0.07	0.00	0.51	0.00	0.06	0.00	0.45	0.00	0.08	0.00	0.53
	Cornflakes	0.14	0.14	0.00	0.00	0.03	0.03	0.00	0.00	0.02	0.02	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
	Corn vermicelli	0.11	0.13	0.70	0.87	0.07	0.08	0.43	0.54	0.06	0.08	0.38	0.48	0.04	0.05	0.27	0.33	0.03	0.04	0.20	0.26
	Ready to eat snacks	**1.62**	**1.74**	**7.97**	**8.53**	**1.05**	**1.11**	**4.79**	**5.13**	**0.31**	**0.32**	**1.70**	**1.82**	**0.05**	**0.05**	**0.00**	**0.00**	**0.05**	**0.05**	**0.00**	**0.00**
	Maize-based snacks	1.57	1.68	7.80	8.35	0.84	0.90	4.41	4.72	0.22	0.24	1.63	1.75	0.03	0.04	0.00	0.00	0.01	0.01	0.00	0.00
	Popcorn	0.05	0.05	0.00	0.00	0.21	0.21	0.00	0.00	0.09	0.09	0.00	0.00	0.01	0.01	0.00	0.00	0.03	0.03	0.00	0.00
	Total	**2.02**	**2.47**	**8.81**	**9.37**	**1.28**	**1.61**	**5.32**	**5.59**	**0.48**	**0.69**	**2.17**	**2.48**	**0.16**	**0.31**	**0.64**	**1.04**	**0.13**	**0.31**	**0.56**	**0.98**
FBs	**Vegetables and fruits**	**0.00**	**0.01**	**0.00**	**0.02**	**0.00**	**0.01**	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.01**
	Fresh corn	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01
	Cereals and cereal products	**2.78**	**2.78**	**19.09**	**19.09**	**3.02**	**3.02**	**19.63**	**19.63**	**2.61**	**2.61**	**14.33**	**14.33**	**1.89**	**1.89**	**11.85**	**11.85**	**2.54**	**2.54**	**13.88**	**13.88**
	Maize kernel	1.31	1.31	4.72	4.72	1.65	1.65	13.06	13.06	1.34	1.34	10.38	10.38	0.80	0.80	5.94	5.94	1.02	1.02	7.98	7.98
	Maize flour	0.03	0.03	0.00	0.00	0.13	0.13	0.00	0.00	0.04	0.04	0.00	0.00	0.02	0.02	0.00	0.00	0.04	0.04	0.00	0.00
	Corn starch	0.01	0.01	0.03	0.03	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
	Corn rice	1.42	1.42	9.70	9.70	1.26	1.26	8.96	8.96	1.22	1.22	9.06	9.06	1.06	1.06	7.92	7.92	1.49	1.49	9.40	9.40
	Cornflakes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Corn vermicelli	0.01	0.01	0.07	0.07	0.01	0.01	0.04	0.04	0.01	0.01	0.04	0.04	0.00	0.00	0.03	0.03	0.00	0.00	0.02	0.02
	Ready to eat snacks	**1.55**	**1.55**	**7.69**	**7.69**	**0.84**	**0.84**	**4.37**	**4.37**	**0.22**	**0.22**	**1.62**	**1.62**	**0.03**	**0.03**	**0.00**	**0.00**	**0.01**	**0.01**	**0.00**	**0.00**
	Maize-based snacks	1.51	1.51	7.51	7.51	0.82	0.82	4.29	4.29	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
	Popcorn	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	**4.33**	**4.34**	**19.27**	**19.27**	**3.86**	**3.87**	**19.72**	**19.72**	**2.83**	**2.83**	**14.38**	**14.38**	**1.93**	**1.93**	**11.88**	**11.88**	**2.56**	**2.56**	**13.90**	**13.90**

Note: **Bold** letters are food groups.

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average body weight of 52.67 kg (Ministry of Health, 2014).

As shown in Table 4, the total mean EDI of DON through maize and maize products ranges from 0.001 to 0.020 µg/kg bw/day for LB and 0.003 to 0.025 µg/kg bw/day for UB scenarios in different age groups. The highest exposure of DON was found in infants and toddlers attaining 0.025 µg/kg bw/day (UB). EDI of the total P95 exposure showed greater values about 3.3 to 6.0 times than the total mean EDI. The total exposure to DON from maize and maize-based food product consumption at the mean and P95 exposure was categorized as low risk due to all the risks shown <100% tolerable daily intake (TDI) (Table 5) ranging from 0.13% to 2.47% (LB and UB) at mean risk and 0.31% to 9.37% (LB and UB) at P95 risk.

Similar to DON, the dietary exposure to FBs through maize and maize-based products was below 100 of its PMTDI of 2 µg/kg bw/day as shown in Table 4. The total mean EDI of FBs through maize and maize products was higher than DON calculated from 0.039 to 0.087 µg/kg bw/day in different age groups. Cereals and cereal product consumption contributed most to the total mean exposure to FBs ranging from 0.038 to 0.061 µg/kg bw/day. In addition, the total exposure at P95 was 4.4 to 6.1 times higher than the total mean exposure, with the highest exposure found in the children's group, with 0.394 µg/kg bw/day (the total mean EDI of 0.077 µg/kg bw/day). The total mean %TDI of FBs (Table 5) in different age groups ranged from 1.93 to 4.34% (LB and UB), while the high risk from P95 attained 11.88% to 19.27 % for LB and UB scenarios. Therefore, the risk of FBs exposure was also categorized as low risk.

4. Discussion

The LOQ value obtained (Table 2) shows a determination of DON and FBs well below EC (EC, 2007) and The National Agency of Drug and Food Control Republic of Indonesia (Indonesian FDA, 2018) maximum limits, as adopted from CAC (2019) for maize-based food products. Also, both RSDr value (precision) and recovery test (accuracy) values were between the required performance criteria for both DON and FBs as established by the EC (2006). Recovery values under repeatability should be less than 60 - 110% for DON and 60 - 120% for FBs, while the RSDr values also should be less than ≤20% for DON and ≤30% for FBs (EU, 2006). The results of the recovery test using spiked samples with a concentration of the spikes ranging from 100 - 500 µg/kg for both DON and FBs were also between the required values established by the EC (2006) which was previously mentioned (results range from 61.18 to 118.66%).

The co-occurrences of DON and FBs were found in 16 of 45 samples, in which FBs occurred more often than DON. Similarly to this present result, Yang *et al*. (2019) reported that 32% of samples (184/576) in maize-based food products in China were contaminated with DON and FBs simultaneously and the highest contamination level in those samples was found in FBs of 9845 µg/kg. However, they reported that DON was found more dominant than FBs in maize food products at 63 and 57% (n = 576), respectively. Kimanya *et al*. (2014) also reported FBs in maize-based products more dominantly occurred than DON at 83 and 44% respectively. Furthermore, 42% of maize food was also found DON and FBs simultaneously which is similar to the present study. The other reports (Lee and Ryu, 2017; Schaarschmidt and Fauhl-Hassek, 2021) and this present study indicate that FBs occur more dominantly in maize than DON despite the fact that both of these toxins can also be found simultaneously.

The highest level of FBs in this study was found in maize flour attaining 400.99 µg/kg, followed by unprocessed or minimally processed maize such as maize kernels (386.66 µg/kg) and corn rice (163.46 µg/kg). The maize processing, which is used as the food is processed either wet milling or dry milling. Dry milling is a physical process which removes the grain's envelope to obtain part of the endosperm to produce products such as corn grits, germs, and flour (Lee *et al*., 2007; Castells *et al*., 2008; Park *et al*., 2018), while wet milling is a maceration process that produces corn starch. According to previous studies conducted by Martins *et al*. (2012) and Escobar *et al.* (2013), the contamination level of FBs in maize which was unprocessed or minimally processed (such as maize kernels) was generally higher than in processed maize. It should be noted; that corn rice is made from minimally processed maize kernels; thus, it may contain FBs in high concentration. In contrast, this current study showed that FBs contamination of maize kernels was lower than maize flour. However, it is well recognized that mycotoxin distribution in maize kernels is not uniform thus it can vary, as well as be affected by other factors such as the origin of maize, climate variation during growth, handling on pre- and post-harvest, and its processing method (Escobar *et al*., 2013).

The low occurrence and low concentration of both DON and FBs in corn vermicelli can be explained by its main ingredient, which is made from corn starch. These findings were in agreement with the results of El-Sayed *et al*. (2003) and Park *et al.* (2018), who explained that DON and FBs might be reduced or eliminated with aqueous fractions in the wet milling process in corn

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starch production as well as the effect of food processing such as extrusion could reduce the contamination levels of mycotoxins in food (Cazzaniga *et al.*, 2001; Castelo *et al.*, 2006; Scudamore and Patel, 2008). Nevertheless, the reduction of mycotoxins during food processing depends on physicochemical characteristics and the initial contamination levels in the raw maize (Wan *et al.*, 2020; Schaarschmidt and Fauhl-Hassek, 2021). It should also be noted that low levels of FBs in cornflakes and maize-based food products can be caused by the binding of FBs to protein, starch and/or polysaccharides as reported by Seefelder *et al.* (2003) and Kim *et al*. (2003), and the FBs bound form, cannot be detected by the methods that were used in this study. The detection of neither mycotoxin in fresh corn can be explained by the low contamination levels in fresh corn as the consequence of the infection of fungi including *F. verticillioides* and *F. graminearum* which is higher during late maturity (Warfield and Gilchrist, 1999; Caldas and Silva, 2007). The contamination of DON and FBs among maize and maize-based food products in this study showed varying levels while significant differences (p<0.01 for Kruskal-Wallis Test) were found on the contamination level of DON and FBs among groups.

Additionally, a similar pattern of the occurrences for DON and FBs in maize and maize-based food products in Indonesia has been reported previously by Ali *et al*. (1998), Setyabudi *et al*. (2012), and Nuryono *et al*. (2004). However, they reported that the contamination level of DON and FBs in the samples was relatively higher than in this current study. DON was found at levels of 47.5 - 348.0 µg/kg (Setyabudi *et al*., 2012), while FBs were found with a range of 12.9 - 2471 µg/kg (Nuryono *et al*., 2014) in maize (maize kernels and maize flour) and maize-based products (extruded maize, popcorn, and fried maize). Jiang *et al*. (2018) reported the contamination levels of DON and FB1 in maize-based products in China were generally higher than this present study ranging from 1.70 - 564.60 g/kg (DON) and 4.16 - 1775 g/kg (FB1) with mean of positive levels of 65.25 and 128.20 g/kg, respectively. The contamination levels of DON and FBs in maize-based products in several tropical countries were also found generally higher than in this present study. As reported in Togo, contamination levels of FBs ranged from 101 - 1838 g/kg in maize intended for consumption (Hanvi *et al*., 2019), while in Tanzania, contamination levels ranged from 64 - 284 g/kg of FBs and 57 - 885 g/kg of DON in maize flour (Kimanya *et al*., 2014) and in Brazil ranged from 2.9 - 1500 g/kg of FBs and 44 - 79 g/kg of DON in maize-based food products (Franco *et al*., 2018).

The contamination level of DON and FBs in this current study showed that none of the samples exceeded the maximum limits of the National regulation (Indonesian FDA, 2018), which is set at 1000 µg/kg in maize and maize-based food products for DON while for FBs is set at 800 µg/kg in maize-based products including cornflakes and 2000 µg/kg for maize flour. The levels also do not exceed the limits established by EC regulations (maximum limits of 750 µg/kg for maize kernels, maize flour and corn starch, corn rice, corn vermicelli; 500 µg/kg for breakfast cereal and maize-based food snacks for DON and 1400 µg/kg for maize kernels and corn rice; 1000 µg/kg for corn vermicelli; 800 µg/kg for cornflakes and maize-based snacks; and 2000 µg/kg for maize flour and corn starch for FBs).

As shown in Table 5, dietary exposure to DON and FBs from maize and maize-based products for both the mean and high exposure (P95) of the calculated values were below 100% of PMTDI of 1 µg/kg bw/day (DON) and 2 µg/kg bw/day (FBs), respectively, which indicates that there is no health risk from either DON and FBs through maize and maize-based product consumption to the Indonesian population. As compared, this current study showed that DON exposure from maize flour was below 0.000 µg/kg bw/day for both children and adults, while FBs exposure was 0.002 and 0.001 µg/kg bw/day for children and adults, respectively, which were substantially lower than the results of both Ji *et al*. (2018) and Mishra *et al*. (2013), who investigated DON exposure through cereal and cereal-based consumption. Furthermore, Ji *et al*. (2018) also revealed that the exposure from wheat flour was higher than maize flour for both Chinese children and adult consumers, while Mishara *et al*. (2012) also showed similar results; the mean EDI value from wheat was almost thirty times higher than maize (3.2 and 0.13 µg/kg bw/day, respectively). Despite the differences in DON contamination levels in cereals, wheat flour was consumed six times more often than maize flour at 172.9 and 28.8 g/day, respectively (Ji *et al*., 2018). In Indonesia, maize is one of the main commodities that is used as food; however, rice is more dominantly consumed (97.7% of the population) and is used as a staple food by almost all regions in Indonesia (Ministry of Health, 2014).

Based on the IFCS (2014) consumption data used in this study, fresh corn was the most common product to be consumed by all respondents (n = 11.980). For information, the consumption data for the eater only, showed that maize kernels and corn rice were consumed with the highest average of 47.51 g/day (n = 1331 respondents) and 46.05 g/day (n = 2354 respondents) (Ministry of Health, 2014). This difference indicates that both maize kernels and corn rice were consumed only by certain populations. According to Suharko (2019), local food products such as corn rice are widely consumed in Central Java, East Java, Madura, and Nusa Tenggara, which makes it a local staple food. The difference in the consumption levels of maize and maize-based food products between all respondents resulted in low exposure to DON and FBs in this study. Also, other maize-based food products were found with a relatively lower amount of consumption which indicates that these foods were only a side food in the daily menu.

In contrast with the present and previous study results that have been mentioned before, high exposure to DON and FBs through maize and maize-based products was found in several countries in Africa. A total of 29% and 46% of all children in Rombo, Northern Tanzania were exposed to DON and FBs at the level of concern of Kimaya *et al*. (2014) while Mahdjoubi *et al*. (2020) also reported high exposure to FBs (mean of 12.91 μg/kg bw/day) through maize consumption in Algeria population. The high exposure of DON and FBs from maize and maize-based products not only caused high contamination of DON and FBs but also the high intake of the maize-based foods in this region (Shephard *et al*., 2013). Dietary exposure to DON from cereal including maize and maize-based products in China shown lower than PMTDI (dietary exposure ranged from 0.41 - 0.49 μg/kg bw/day for children and 0.22 - 0.26 μg/kg bw/day for adults), suggesting no health risk for children and adults' population at mean level (Sun and Wu, 2017). In South Asia, a study of dietary intake to FBs through maize was investigated in Vietnam, where the findings showed that mean FBs exposure was also lower than its PMTDI. However, the authors note for future reference that the P95 was higher by 1.1 to 1.9 times than the PMTDI (Huong *et al*., 2016; Do *et al.*, 2020). It is well known that mycotoxins exposure in humans depends on the level of contamination as well as the intake of the food. For further information, the highest exposure of DON and FBs in nine different samples was found almost entirely in the infant and child groups. This can be explained by infants, toddlers and children having higher food consumption per kg body weight; therefore, this requires special attention considering that infants, toddlers, and children are vulnerable due to their sensitivity to some neurotoxic, endocrine, and immunological effects (Boon *et al.*, 2009).

As with the majority of studies, the design of the current study is subject to limitations. Exposure assessment is associated with uncertainties that need to be considered to understand correctly the strength and limitations of its results (Kettler *et al*., 2015). In this study, exposure assessment of DON and FBs are based on a point estimate approach which does not take into account the variabilities and uncertainties of differences in food consumption between populations in Indonesia, which could contribute to an under- or overestimation of food consumption and further affect the exposure assessment. Extrapolation of contamination levels of DON and FBs zone data to Indonesia as a whole also could raise uncertainty which might influence the overview of contamination levels and lead to exposure becoming lower or considerably higher in a particular region.

5. Conclusion

In conclusion, co-occurrences of DON and FBs were found at 35.56% in maize and maize-based food, while FBs were the most abundant toxin with an occurrence level of 84.88%. Both DON and FBs were reported at a lower level than the maximum limits established by the Indonesian FDA and the EC. Estimated daily intake of DON and FBs were not considered to be a health concern due to, the mean and high exposure not being above 100% of the Provisional Maximum Daily Intake of 1 μg/kg bw/day for DON and 2 μg/kg bw/day for FBs. However, the highest exposure to DON and FBs was found in vulnerable groups such as infants, toddlers, and children, so an in-depth study of these groups is needed.

Conflict of interest

The authors declare no conflict of interest.

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