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Study on Contamination with Some Mycotoxins in Maize and Maize-Derived Foods

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Abstract: Crops can be contaminated by fungi which produce mycotoxins. Many fungal strains are responsible for producing varied mycotoxins. The research carried out so far has described over 400 different mycotoxins. They have chemical and physical properties that significantly differ, and they are produced by several different existing fungi. The intake of mycotoxins through food can be achieved directly, by feeding on contaminated food, or indirectly from foods of animal origin. The mycotoxin contamination of food and food products for certain animals is a phenomenon studied worldwide, in countries in Europe but also in Asia, Africa and America. The purpose of this study is to develop an evaluation of the mycotoxins prevalent in corn and corn-derived products produced in Romania. A total of 38 maize samples and 19 corn-derivative samples were investigated for the presence of mycotoxins specific to these products, such as deoxynivalenol, zearalenone and fumonisins. Fumonisins had the highest presence and zearalenone had the lowest. The limits determined for the three mycotoxins were always in accordance with legal regulations.

Keywords: corn; deoxynivalenol; food; fumonisins; mycotoxins; zearalenone

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1. Introduction

Corn (*Zea mays* L.) is native to Central America, cultivated worldwide as a food, industrial and fodder plant and was first mentioned in Romania in the late seventeenth century. Maize is a plant of the *Poaceae* family. The annual harvest of maize in 2020 was 1192 million tons, as maize is one of the most commonly used cereals in food [1]. Mycotoxins infect corn in the field or during the storage stage [2]. Mycotoxin contamination is the main problem with maize production. Mycotoxins are formed by filamentous fungi of the genus *Ascomycota*, which affects human and animal health and causes chronic and acute diseases [3–6]. Corn is most frequently contaminated by *Aspergillus* sp. and *Fusarium* sp. [7].

The most important mycotoxins that contaminate maize throughout its development cycle and storage period are fumonisins (FUM), deoxynivalenol (DON) and zearalenone (ZEA) [8]. Fumonisins are formed by *Fusarium verticillioides*, *F. proliferatum* and *F. fujikuroi*; zearalenone are formed by *Fusarium graminearum*, *F. culmorum*, *F. cerealis* and *F. equiseti*; deoxynivalenol are produced by *Fusarium graminearum* and *F. culmorum* [9]. The development of fungi and, by extension, mycotoxins, is favored by high temperatures, high humidity and prolonged storage [10]. The possibility of mycotoxin production rises if inappropriate agricultural practices are applied.

Mycotoxins cannot be detected in the visible field (VIS) but can be recognized in ultraviolet (UV) light; moreover, they do not have a distinctive smell and do not change the organoleptic properties of foodstuff [11]. The consumption of corn contaminated with mycotoxins by humans or animals can generate multiple serious toxic effects [12]. Exposure to mycotoxins leads to mycotoxicosis (ergotism, food poisoning and aflatoxins), a disease affecting human organs which, under certain conditions, may cause death [11,13].

Mycotoxicosis may be acute or chronic [2]. The main ways of exposing the human body to mycotoxins are by ingestion, inhalation and contact.

The European Union has established by legislation limits on the content of mycotoxins in all categories of foodstuffs, including corn and corn-derived aliments, to protect the population's health. Regulation (EC) No. 1881/2006 [14] sets the maximum levels for aflatoxins, fumonisins, zearalenone and deoxynivalenol in food. This regulation was amended by (EC) Regulation No. 1126/2007 [15] and (EC) Regulation No. 165/2010 [16]. Prevention, detoxification and decontamination measures are needed to combat mycotoxin contamination of food [17]. The objective of this study is to assess the contamination with three mycotoxins, namely deoxynivalenol, zearalenone and fumonisins, of maize and maize-derived products from local producers in the eastern part of Romania.

2. Materials and Methods

2.1. Biological Material

For the study of the deoxynivalenol, zearalenone and fumonisin contamination of corn and corn-derived foods, 57 samples were taken from the Romanian market, from the local producers of corn or corn products (breakfast cereals, cornflakes, canned corn, puffs and puff pastry). The grain maize samples were taken from various producers in eastern Romania. Samples were taken after the harvesting and drying of corn kernels and before storing them in silos. A total of 38 samples of grain maize were analyzed: 16 samples for the detection of deoxynivalenol, 12 samples for the detection of zearalenone and 10 samples for the investigation of fumonisins. The samples of food products derived from corn were taken from local producers in the eastern part of Romania and all products were autochthonous. A total of 19 samples were analyzed: 5 samples for the detection of deoxynivalenol (2 samples of corn flakes and 3 samples of maize breakfast cereals), 10 samples for the investigation of zearalenone (3 samples of preserved maize, 3 samples of corn flakes, 3 samples of maize breakfast cereals and 1 sample puffed) and 4 samples for the detection of fumonisins (2 canned maize samples and 2 corn flakes samples).

2.2. Sampling Method

Mycotoxins are distributed unevenly in a product, so every measure was taken at sampling so to ensure that the sample was representative. In the case of the grain maize samples, a number of 100 incremental samples were taken, making up the 10 kg aggregate sample. For products derived from maize, 3 incremental samples were taken each, making up the aggregate sample, weighing 1 kg. The analysis of the samples was made in one replication. When selecting a sample for the determination of a particular mycotoxin, the evaluations performed by each economic operator in the previous years regarding the risk of mycotoxins were taken into account, while also taking into account the mycotoxin most frequently identified in the previous years.

2.3. Laboratory Method

ELISA (enzyme-linked immunosorbent assay) kits provided by ProGnosis Biotech S.A were utilized to analyze the content of deoxynivalenol, zearalenone and fumonisins in the samples. The kits conformed to the specifications of EN ISO 14675: 2003. ProGnosis Biotech S.A is ISO 9001: 2015 was certified by TÜV Hellas (TÜV NORD). Bio-Shield deoxynivalenol, B2648/B2696, Bio-Shield zearalenone, B2748/B2796 and Bio-Shield fumonisin, B2848/B2896, are ELISA tests used to establish the content of deoxynivalenol, zearalenone and fumonisins in food and animal feed [18]. Other materials utilized include a grinding device, balance, graduated cylinder, distilled water, filter paper, filter funnel, laboratory tubes, micropipettes, mixer and spectrophotometer. The aggregate sample was homogenized and ground in the laboratory. The toxins were extracted from the sample ground with distilled water; the sample was then ground to obtain fine particles weighing 20 g and then added to 100 mL of distilled water. The mixture obtained was homogenized with the mixer, filtered and diluted 5 times with distilled water. The standards of the mycotoxin or the samples, and the detection solution, were added to padded wells. The enzyme conjugate and antimycotoxin antibodies were added to each well and incubated for 5 min at room temperature. Discard the liquid from the wells, add the wash buffer, then incubate the chromogenic substrate for 3 min in the dark at room temperature. The conjugate connects to the antibody, and the accession places padding not already occupied by mycotoxin in standards or samples. The addition of a chromogen substrate leads to the appearance of a blue complex. Sulfuric acid must be added, which stops the development of the color, which becomes yellow. The photometric reading was made at 450 nm [19–21]. The competitive format of the ELISA was the most used [22].

2.4. Statistical Analysis

All results are presented as mean standard deviation. The values obtained were processed by using the SPSS 25.0 (trial version) software (IBM, New York, NY, USA). The difference between the samples was established by an analysis of variance (ANOVA) using the Turkey's test at a 5% significance level.

3. Results

3.1. Deoxynivalenol

Deoxynivalenol (Figure 1) is also named vomitoxin and it is produced by the *Fusarium* species, which is adapted to various pedoclimatic conditions, and found in Romania in the plateaus of Moldova and Dobrogea or in the wetlands of the Danube Delta. *Fusarium graminearum* can infect maize and wheat, but it was also isolated from rice, coffee, peas and tomatoes [23,24]. *Fusarium culmorum* can infect plants of the *Gramineae* family (wheat, rye, rice, oats and corn) but also plants of the *Solanaceae*, *Leguminosae* and *Cucurbitaceae* family. Deoxynivalenol is one of the least toxic of the trichothecenes, and its appearance is common, but in combination with other mycotoxins (fumonisins, T2 toxin and beauvericin) it can have much more serious effects [25].

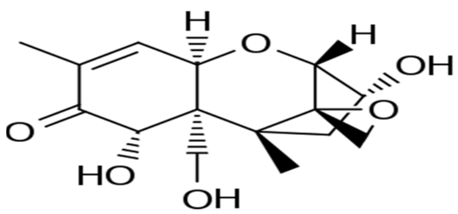

Figure 1. Deoxynivalenol chemical structure.

Deoxynivalenol is a very stable trichothecene at high temperatures of 120 °C, and less stable at 180 °C. It may be dissolved in water, ethanol, methanol, chloroform, acetonitrile and ethyl acetate [26].

Analyzing the results presented in Table 1 and Figures 2 and 3, we can say that in 25% of the analyzed samples of corn grains, deoxynivalenol was identified in 20% of the samples of corn products. The determined values do not exceed the limits established by Regulation (EC) No. 1881/2006 with the subsequent modifications (for maize grains, the maximum of 1750 µg/kg, and for maize-derived foods, the maximum of 500 µg/kg). The deoxynivalenol level is 200 µg/kg in cereal-based aliments for children, 750 µg/kg in pasta and 500 µg/kg in bread [24]. The EFSA recently established a PMTDI of 1 µg/kg bw/day for deoxynivalenol and its metabolites [27,28].

Table 1. Study on the incidence of deoxynivalenol in maize and maize-derived foods in Romania.

Sample Number	The Analyzed Product	Country of Origin	Limit of Detection µg/kg	Limit of Quantification µg/kg	Deoxynivalenol µg/kg	Limit µg/kg
1.	Maize01	Romania	40.720	67.198	<67.198 [e]	1750
2.	Maize02	Romania	40.720	67.198	70.400 [d]	1750
3.	Maize03	Romania	40.720	67.198	<40.720 [g]	1750
4.	Maize04	Romania	40.720	67.198	77.100 [c]	1750
5.	Maize05	Romania	40.720	67.198	<40.720 [g]	1750
6.	Maize06	Romania	40.720	67.198	<40.720 [g]	1750
7.	Maize07	Romania	40.720	67.198	<67.198 [e]	1750
8.	Maize08	Romania	40.720	67.198	<67.198 [e]	1750
9.	Maize09	Romania	40.720	67.198	<40.720 [g]	1750
10.	Maize10	Romania	40.720	67.198	92.900 [b]	1750
11.	Maize11	Romania	40.720	67.198	<40.720 [g]	1750
12.	Maize12	Romania	40.720	67.198	152.200 [a]	1750
13.	Maize13	Romania	40.720	67.198	<40.720 [g]	1750
14.	Maize14	Romania	40.720	67.198	<40.720 [g]	1750
15.	Maize15	Romania	40.720	67.198	<40.720 [g]	1750
16.	Maize16	Romania	40.720	67.198	<67.198 [e]	1750
17.	Cornflakes01	Romania	18.610	37.230	<18.610 [h]	500
18.	Cornflakes02	Romania	18.610	37.230	<18.610 [h]	500
19.	Breakfast cereals01	Romania	18.610	37.230	<18.610 [h]	500
20.	Breakfast cereals02	Romania	18.610	37.230	63.990 [f]	500
21.	Breakfast cereals03	Romania	18.610	37.230	<18.610 [h]	500

The differences between samples were established by analysis of variance (ANOVA) using Turkey's test at a 5% significance level. Different superscript letters after the values indicated a statistically significant difference at $p < 0.05\%$.

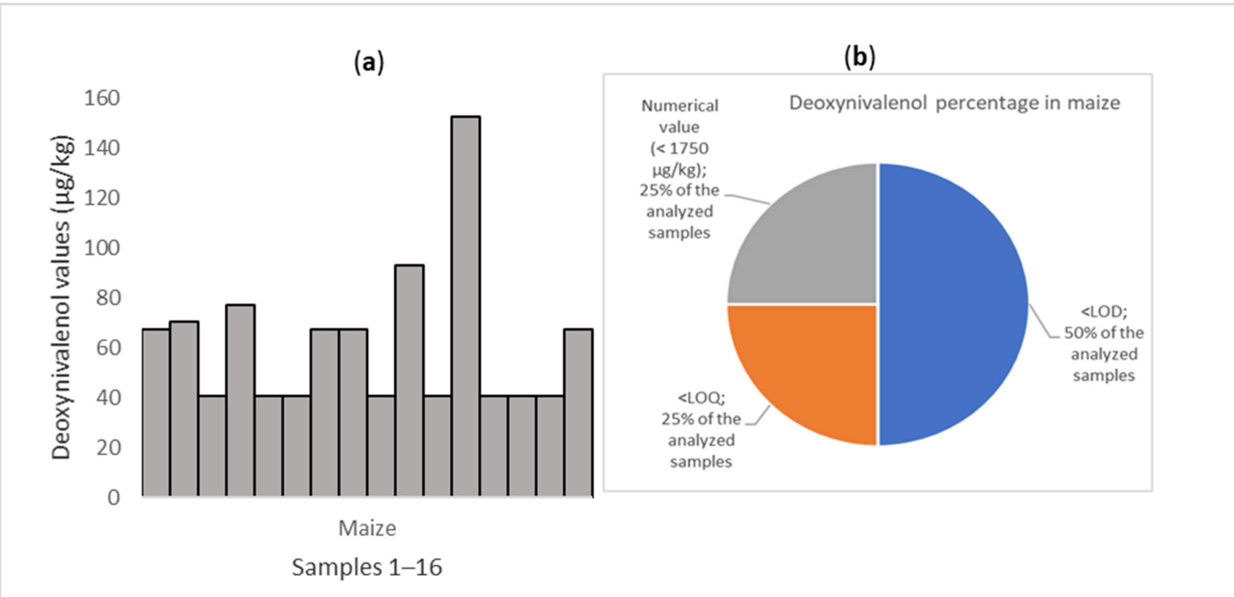

Figure 2. (**a**) Deoxynivalenol in maize samples; (**b**) Deoxynivalenol percentage in maize samples.

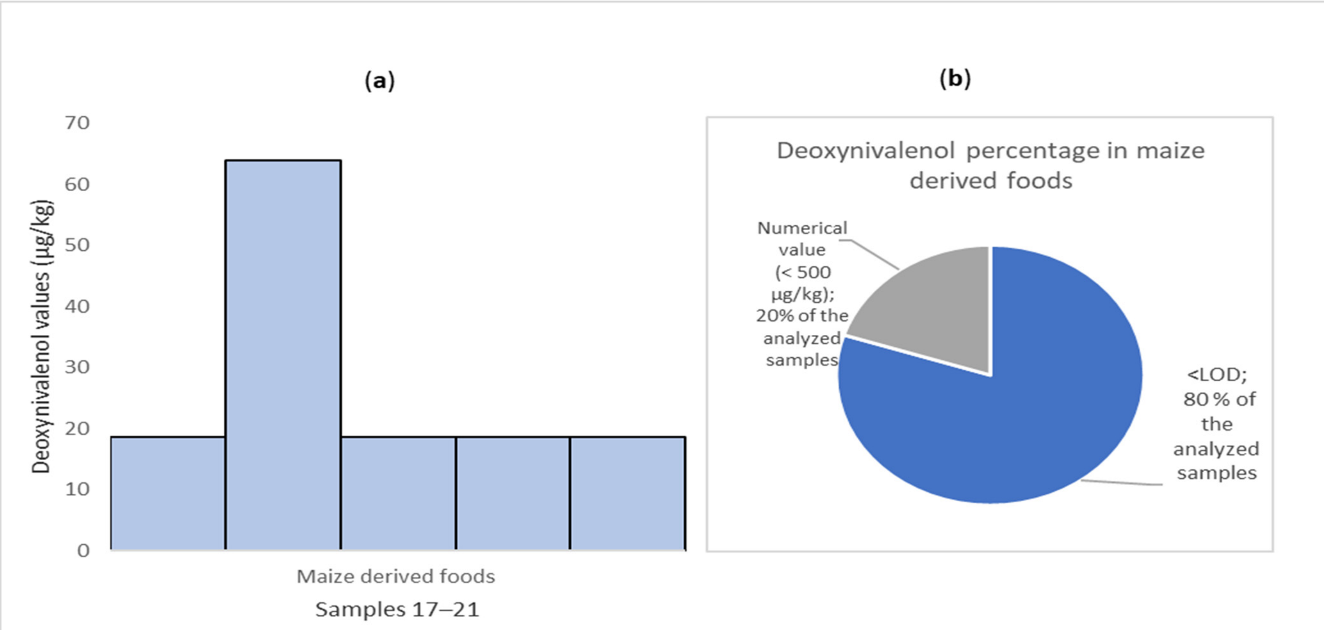

Figure 3. (**a**) Deoxynivalenol in maize-derived food samples; (**b**) Deoxynivalenol percentage in maize-derived food samples.

The difference between the samples analyzed was significantly different (95% confidence level). In the maize category, the results obtained for samples 1, 7, 8 and 16 are not significant. The same was observed for samples 3, 5, 6, 9, 11, 13, 14 and 15. On the other hand the results obtained for the remaining four samples (samples 2, 4, 10, 12) are significantly different ($p < 0.5\%$). The two cornflakes samples presented similar results. From the three breakfast cereals studied, only one (sample 2) was found to be significantly different (at 95% confidence level).

Deoxynivalenol is the most commonly identified mycotoxin in cereals, with a mean percentage between 50% and 76% in Asia and Africa. Higher concentrations have been identified in Europe and Asia [29]. In North America, the reported deoxynivalenol levels are similar to those in the rest of the world. Increased levels of deoxynivalenol are recorded in years of severe infections caused by *Fusarium* sp. [30].

Exposure to deoxynivalenol is usually made by aliments. The intake of contaminated foodstuffs may cause nausea, vomiting, inappetence and diarrhea. When foodstuffs consumed daily, for a longer period of time, are contaminated by deoxynivalenol, manifestations such as weight decline, anorexia and histopathological lesions in the liver occur [23]. In India, the intake of contaminated food has led to acute gastric symptoms, vomiting and diarrhea [4]. There is no evidence for the carcinogenic potential of deoxynivalenol, which is why the IARC classifies this mycotoxin as noncarcinogenic to humans (Group 3) [31].

For the deoxynivalenol parameter in samples in Romania, the determined values, compared to other studies in other parts of the globe, as presented in Table 2, are low. Moreover, the incidence in food samples is reduced.

Table 2. Studies on the incidence of deoxynivalenol in maize and maize-derived foods.

Country	The Analyzed Product	No. Samples	Deoxynivalenol Incidence%	Deoxynivalenol Mean µg/kg	Deoxynivalenol Range µg/kg	Reference
Romania	Corn	16	25.00	61.70	n/a–152.20	
	Corn flakes, Breakfast cereals	5	20.00	27.69	n/a–63.99	

Table 2. *Cont.*

Country	The Analyzed Product	No. Samples	Deoxynivalenol Incidence%	Deoxynivalenol Mean µg/kg	Deoxynivalenol Range µg/kg	Reference
Serbia	Corn flakes	15	40.00	255.00	n/a–878.00	[32]
	Corn flour	56	42.90	101.00	n/a–931.00	[32]
Hungary	Corn	29	86.00	1872.00	225.00–2963.00	[33]
	Corn	106	92.30	449.00	263.10–2777.40	[34]
Belgium	Corn	106	100.00	557.50	337.40–5322.40	[34]
	Corn	106	64.70	186.50	121.30–2110.50	[34]
Germany	Corn	120	100.00	216.50	n/a–1097.20	[35]
Switzerland	Corn	19	100.00	135.60	78.00–299.00	[36]
China	Corn	44	65.90	831.00	5.80–9843.30	[37]
Brazil	Pastry	36	100.00	591.00	60.00–1720.00	[38]

3.2. Zearalenone

Fusarium fungi produce zearalenone (Figure 4), and the main species producing zearalenone are *F. roseum*, *F. tricinctum*, *F. oxysporum*, *F. graminearum*, *F. moniliforme*, *F. culmorum*, *F. avenaceum*, *F. crookwellense*, *F. nivale*, *F. semitectum*, *F. solani* and *F. echiseti*, which are spread throughout the world, especially in temperate climates [1,39]. Zearalenone infection is reduced in cereals at harvest and raises in storage conditions if humidity reaches 30–40% [1]. Zearalenone is stable at high temperatures (80 °C–120 °C) and in neutral media. It may be dissolved in methyl chloride, dimethylformamide alcohols and ethers [40].

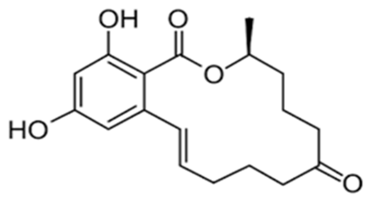

Figure 4. Zearalenone chemical structure.

The maximum levels of zearalenone are established by Regulation (EC) 1881/2006 for several food categories: 350 µf/kg maize, other cereals 100 µg/kg, bread, pastry, breakfast cereals 50 µg/kg and food for infants 20 µg/kg [41–43].

From all the maize samples, only 12 are significantly different ($p < 0.05\%$). No significant difference was found at a 95% confidence level between the cornflakes, breakfast cereals and puffs samples. Moreover, the results obtained showed no significant difference ($p < 0.05\%$) for the maize and canned corn samples.

The study on the presence of zearalenone was performed on 22 samples of corn grains and corn products (canned corn, corn flakes, breakfast cereals and puff pastry) produced in Romania, as presented in Table 3. Of the 12 corn samples analyzed, 8% were contaminated with zearalenone, and none of the samples of the maize-based foods were contaminated with zearalenone. The determined values did not exceed the maximum limit established by Regulation (EU) No. 1881/2006 (for maize grains, the maximum of 350 µg/kg and for food based on maize, the maximum of 50–100 µg/kg), as presented in Figures 5 and 6.

Table 3. Study on incidence of zearalenone in maize and maize-derived foods in Romania.

Sample Number	The Analyzed Product	Country of Origin	Limit of Detection µg/kg	Limit of Quantification µg/kg	Zearalenone µg/kg	Limit µg/kg
1.	Maize01	Romania	2.060	2.456	<2.060 ^{b}	350
2.	Maize02	Romania	2.060	2.456	<2.060 ^{b}	350

Table 3. *Cont.*

Sample Number	The Analyzed Product	Country of Origin	Limit of Detection µg/kg	Limit of Quantification µg/kg	Zearalenone µg/kg	Limit µg/kg
3.	Maize03	Romania	2.060	2.456	<2.060 [b]	350
4.	Maize04	Romania	2.060	2.456	<2.060 [b]	350
5.	Maize05	Romania	2.060	2.456	<2.060 [b]	350
6.	Maize06	Romania	2.060	2.456	<2.060 [b]	350
7.	Maize07	Romania	2.060	2.456	<2.060 [b]	350
8.	Maize08	Romania	2.060	2.456	<2.060 [b]	350
9.	Maize09	Romania	2.060	2.456	<2.060 [b]	350
10.	Maize10	Romania	2.060	2.456	<2.060 [b]	350
11.	Maize11	Romania	2.060	2.456	<2.060 [b]	350
12.	Maize12	Romania	2.060	2.456	18.565 ± 1.411 [a]	350
13.	Canned corn01	Romania	2.060	2.456	<2.060 [b]	100
14.	Canned corn02	Romania	2.060	2.456	<2.060 [b]	100
15.	Canned corn03	Romania	2.060	2.456	<2.060 [b]	100
16.	Cornflakes01	Romania	1.860	3.730	<1.860 [c]	100
17.	Cornflakes02	Romania	1.860	3.730	<1.860 [c]	100
18.	Cornflakes03	Romania	1.860	3.730	<1.860 [c]	100
19.	Breakfast cereals01	Romania	1.860	3.730	<1.860 [c]	50
20.	Breakfast cereals02	Romania	1.860	3.730	<1.860 [c]	50
21.	Breakfast cereals03	Romania	1.860	3.730	<1.860 [c]	50
22.	Puffs01	Romania	1.860	3.730	<1.860 [c]	100

The differences between samples were established by analysis of variance (ANOVA) using Turkey's test at a 5% significance level. Different superscript letters after the values indicated statistically significant differences at $p < 0.05\%$.

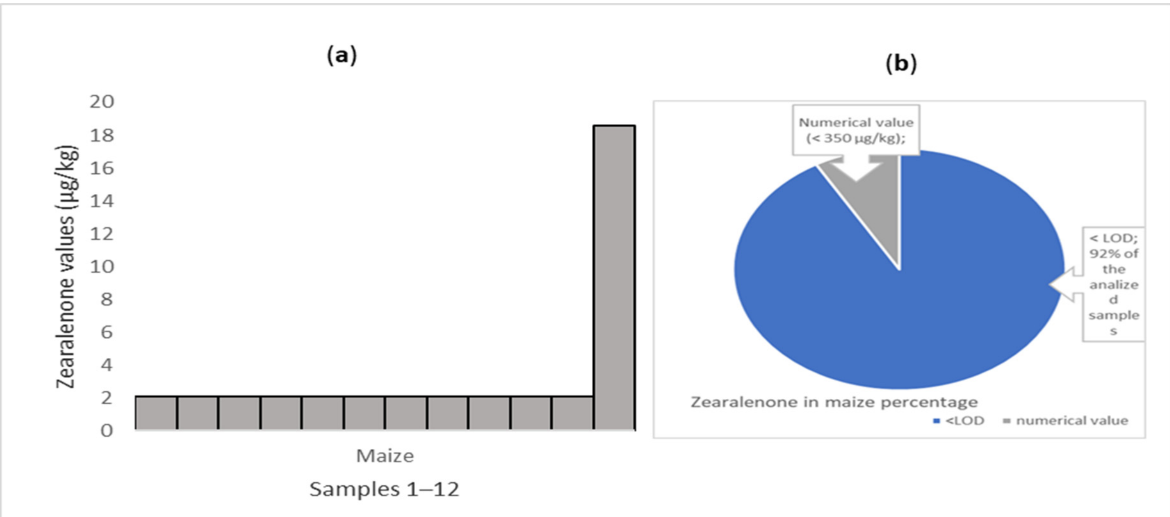

Figure 5. (**a**) Zearalenone in maize samples; (**b**) Zearalenone percentage in maize.

Risk assessments for the intake of foods contaminated with zearalenone have been performed based on existing exposure data from countries in Europe and Asia. Studies have identified several situations where the daily intake of zearalenone exceeds the TDI as stated by the European Union [43]. According to the IARC, zearalenone is a Group 3 carcinogen [34]. Zearalenone is the lactone of resorcin acid whose chemical structure resembles that of steroid hormones. Zearalenone has nonsteroidal estrogenic action and affects the conception, ovulation and development of the fetus at concentrations over 1 mg/kg [44]. Zearalenone can cause hyperestrogenism and it primarily affects reproductive functions.

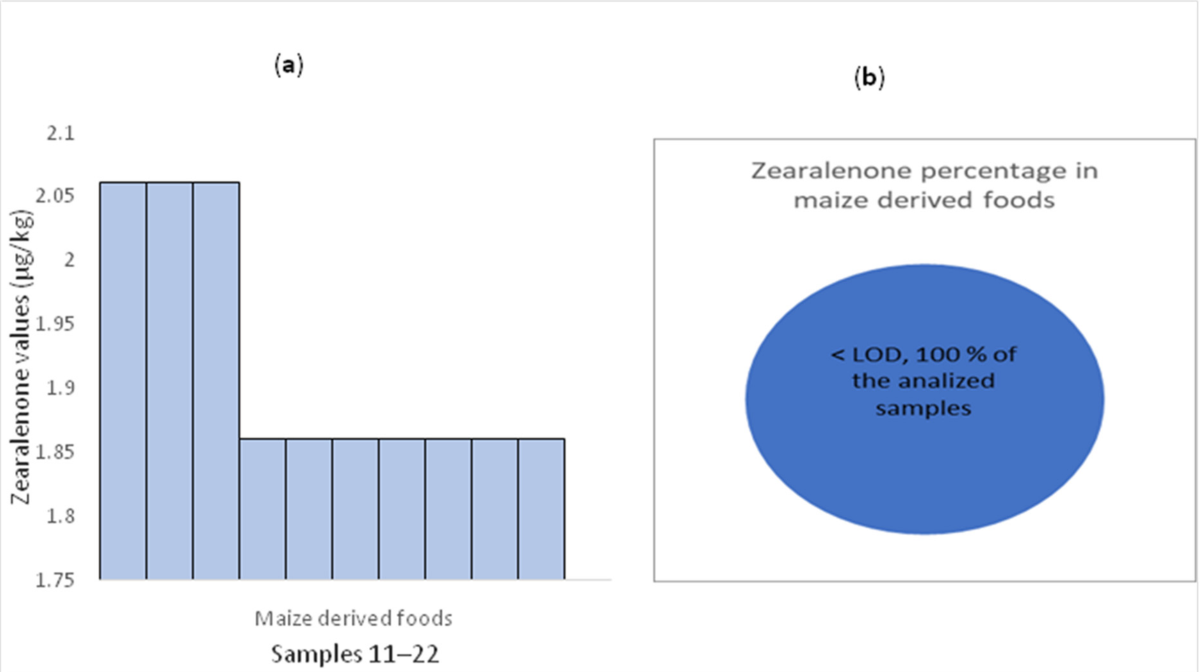

Figure 6. (**a**) Zearalenone in maize-derived food samples; (**b**) Zearalenone percentage in maize-derived food samples.

Table 4 shows the specific studies on the presence of zearalenone in maize and maize-derived foods worldwide from 2014 to 2020. The determined values, compared to other studies in other areas of the globe, as presented in Table 4, are low. Moreover, the incidence in food samples is reduced.

Table 4. Studies on the incidence of zearalenone in maize and maize-derived foods.

Country	The Analyzed Product	No. Samples	Zearalenone Incidence%	Zearalenone Mean µg/kg	Zearalenone Range µg/kg	Reference
Romania	Corn	12	8.00	3.44	nd–18.565	
	Corn flakes, breakfast cereals, canned corn, puffs	10	0.00	nd	nd	
Serbia	Maize	90	0.00	0.00	0.00	[45]
Hungary	Maize	29	41.00	267.00	nd–565.00	[33]
	Maize	106	64.80	100.50	71.20–1085.60	[34]
Belgium	Maize	106	40.70	158.50	0.00–1085.60	[34]
	Maize	106	42.40	175.50	0.00–2791.60	[34]
Germany	Maize	120	96.00	819.00	nd–3910.00	[35]
Brazil	Maize	40	95.00	22.20	1.80–99.00	[46]
Egypt	Maize	30	33.34	2.39	1.13–3.70	[47]
	Maize	50	94.00	109.10	0.20–3613.00	[35]
China	Maize	44	13.60	50.80	40.70–1056.80	[48]

3.3. Fumonisins

Fumonisins (Figures 7 and 8) are also called *Fusarium* toxins. The main producing species for fumonisins are *Fusarium verticillioides*, *F. proliferatum*, *F. sacchari*, *F. fujikuroi* and

F. subglutinans. [49]. The twenty-eight fumonisin analogs identified so far are split into four groups: fumonisins A, B, C and P. Group Fumonisins B includes FB1, FB2 and FB3. Of these, FB1 is the most frequent and has the highest concentrations [50]. Fumonisins have been identified in several food categories including cereals [51], foods derived from cereals [52,53], asparagus, garlic [54], grapes, beer, milk and raisins [55].

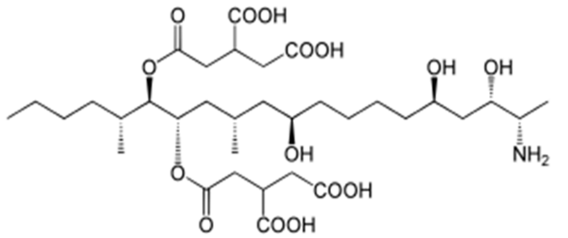

Figure 7. Fumonisin B1 chemical structure.

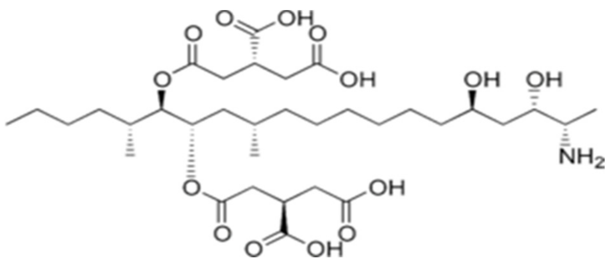

Figure 8. Fumonisin B2 chemical structure.

The study on the presence of fumonisins was performed on 14 samples of maize and maize-derived foods (canned corn and corn flakes) produced in Romania, as presented in Table 5.

Table 5. Study on incidence of fumonisins in corn and corn-derived foods in Romania.

Sample Number	The Analyzed Product	Country of Origin	Limit of Detection µg/kg	Limit of Quantification µg/kg	Fumonisins µg/kg	Limit µg/kg
1.	Maize01	Romania	21.722	30.644	163.551 ± 49.065 [b]	4000
2.	Maize02	Romania	21.722	30.644	1009.360 ± 302.804 [a]	4000
3.	Maize03	Romania	21.722	30.644	38.318 ± 11.495 [f]	4000
4.	Maize04	Romania	21.722	30.644	<21.722 [h]	4000
5.	Maize05	Romania	21.722	30.644	<21.722 [h]	4000
6.	Maize06	Romania	21.722	30.644	<21.722 [h]	4000
7.	Maize07	Romania	21.722	30.644	45.614 ± 13.684 [e]	4000
8.	Maize08	Romania	21.722	30.644	104.368 ± 31.315 [c]	4000
9.	Maize09	Romania	21.722	30.644	79.824 ± 23.947 [d]	4000
10.	Maize10	Romania	21.722	30.644	39.260 ± 12.640 [f]	4000
11.	Canned corn01	Romania	25.900	51.810	<25.900 [g]	1000
12.	Canned corn02	Romania	25.900	51.810	<25.900 [g]	1000
13.	Cornflakes01	Romania	25.900	51.810	73.000 ± 5.590 [d]	800
14.	Cornflakes02	Romania	25.900	51.810	<25.900 [g]	800

The differences between samples were established by analysis of variance (ANOVA) using Turkey's test at a 5% significance level. Different superscript letters after the values indicate statistically significant differences at $p < 0.05\%$.

From all the maize samples, samples 4, 5 and 6 are not significantly different ($p < 0.05\%$). All the other samples are significantly different from each other. No significant (at 95% confidence level) difference was found between the canned corn samples. The result obtained for the two cornflakes samples showed a significant difference ($p < 0.05\%$) between them.

The mycotoxins identified in this research, in the largest percentage of the analyzed samples (70%), were fumonisins, as presented in Figures 9 and 10. The determined values are within the allowed legal limits. In 70% of the samples of unprocessed corn, fumonisins were detected; in those of products processed from corn, only 25% indicated their presence.

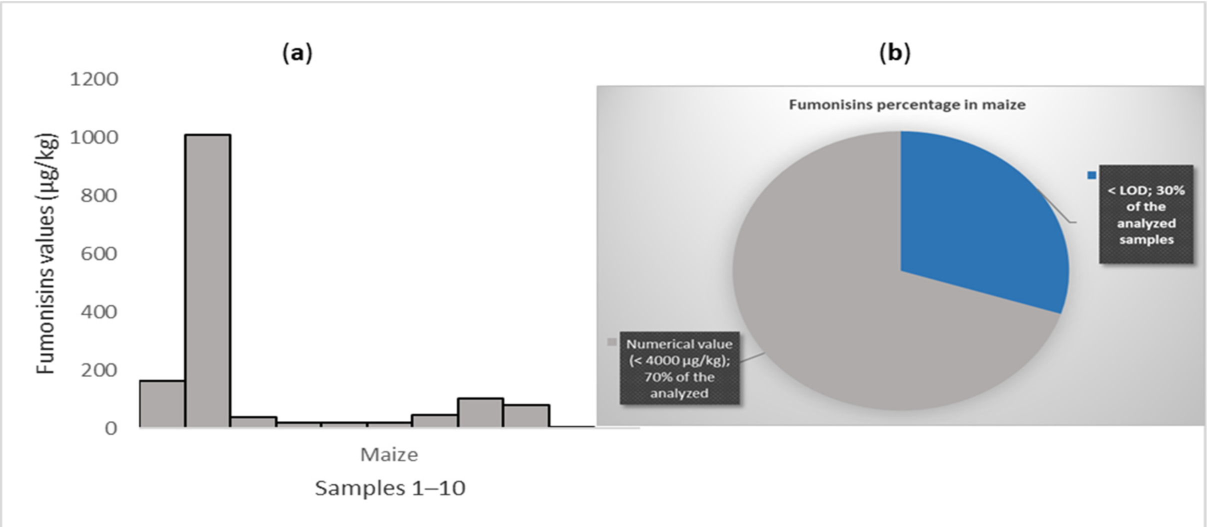

Figure 9. (**a**) Fumonisins in maize samples; (**b**) Fumonisins percentage in maize.

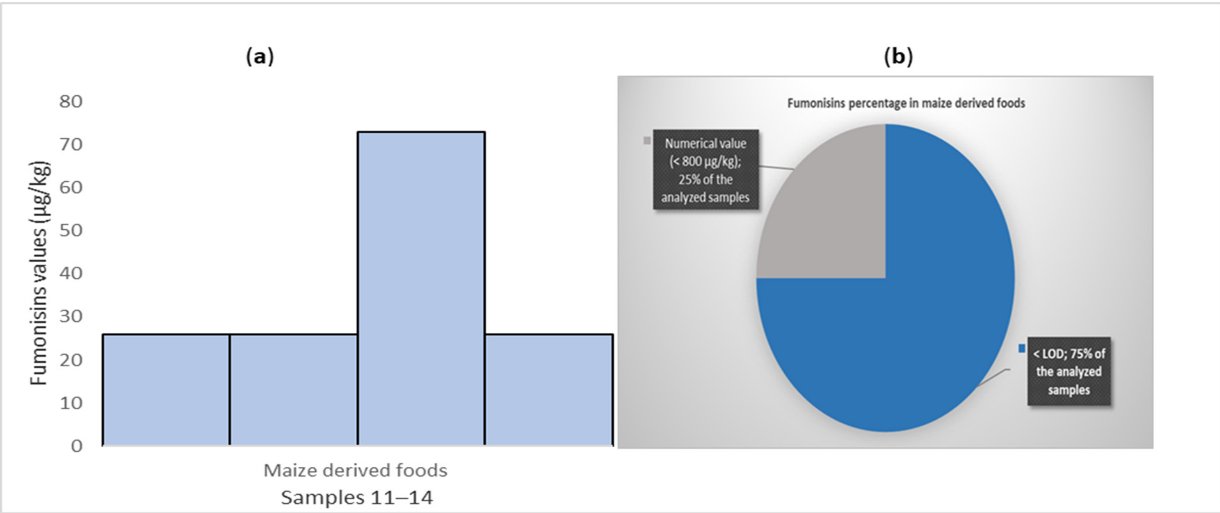

Figure 10. (**a**) Fumonisins in maize-derived foods; (**b**) Fumonisins percentage in maize-derived foods.

JECFA has provisionally settled a PMTDI of 2 µg/kg bw/day for fumonisins alone or in combination [56]. The maximum levels of fumonisins are set by Regulation (EC) 1881/2006 for several food categories: 4000 µg/kg for maize, 800 µg/kg for breakfast cereals and 200 µg/kg for food for children [57].

The fumonisin values from the samples investigated, compared to samples from other areas of the globe, as shown in Table 6, are higher. The impact on the food samples in Romania is also higher.

Table 6. Studies on the occurrence of fumonisins in maize and maize-derived foods.

Country	The Analyzed Product	No. Samples	Fumonisins Incidence%	Fumonisins Mean µg/kg	Fumonisins Range µg/kg	Reference
Romania	Corn, canned corn, corn flakes	10	70.00	154.55	nd–1009.36	
		4	25.00	37.68	nd–73.00	
Serbia	Corn	90	100.00	1730.00	520.00–5800.00	[45]
Belgium	Corn	106	2.50	1.50	0.00–70.20	[34]
	Corn	106	19.80	61.10	0.00–1362.90	
	Corn	106	61.20	247.40	54.00–4414.90	
	Corn	106	nd	nd	nd	
	Corn	106	4.90	9.00	0.00–412.60	
	Corn	106	24.70	61.60	0.00–1427.40	
	Corn	106	nd	nd	nd	
	Corn	106	7.40	3.40	0.00–90.50	
	Corn	106	18.80	18.00	0.00–451.20	
	Corn	106	2.50	1.30	0.00–70.20	
	Corn	106	19.80	73.60	0.00–1782.80	
	Corn	106	61.20	327.00	58.70–6293.50	
Italy	Corn	697	100.00	10900.00	25.00–77000.00	[58]
Brazil	Corn	40	100.00	2338.50	230.00–6450.00	[46]
Spain	Corn	92	100.00	2610.00	337.00–10613.00	[59]
Spain	Corn flakes	47	21.00	42.00	nd–67.00	
Canada	Corn	10	100.00	4.64	0.73–10.21	[60]
China	Corn flakes	14	100.00	104.10	1.00–171.00	[61]
	Corn flakes	14	93.00	14.20	<0.27–25.60	
	Corn flakes	14	93.00	17.30	<0.27–31.50	

4. Discussion

Strategies to prevent mycotoxin contamination prior to harvesting are particularly important. Good agricultural practices and good production practices are used by producers around the world. Crop rotation, the use of approved substances such as herbicides, fungicides and insecticides (insects are vectors for fungal dissemination) and storage in appropriate conditions for temperature and humidity contributes substantially to reducing the risk of contamination of cereals in the first stages of the food chain [62,63]. The implementation of HACCP (hazard analysis and critical control points), through clear instructions for monitoring the presence and level of mycotoxins in food, allows safe foods to be placed on the market.

One of the greatest dangers associated with food safety is mycotoxins. They have negative effects on all biological systems, from affecting clinical conditions, cellular systems and the metabolism, to decreased animal production. Mycotoxins cause major economic losses through direct action, inducing disease indirectly through damage to the immune system, or qualitatively affecting contaminated products.

The world's need for goods commonly used in the manufacture of food for humans and animals, such as corn, has grown constantly in the last years due to higher demand from manufacturers and consumers. The RASFF annual report (Rapid Alert System for Food and Feed) for 2020 presents 400 notifications regarding mycotoxin (down by 23%) contamination of food products in the European Union [64]. Aflatoxins are the most detected mycotoxins in food in the EU (367 notifications), also detected in dried figs from Turkey (58 notifications) and followed by groundnuts from the United States (29 notifications) [64]. The formation of mycotoxins is a composite process influenced by a multitude of factors, mainly environmental conditions under the influence of climate change.

Comparing the results of this study with previous research, for deoxynivalenol, the incidence in the analyzed samples is 25% for maize, while in Hungary the incidence of deoxynivalenol is 86% [33]; in Belgium, the incidence in the studies carried out varies from 64.7% to 92.3% or 100% [34]. Studies carried out in Germany and Switzerland show a deoxynivalenol incidence of 100%, while in China the incidence is 65.9% [35–37]. For the analyzed products derived from maize, the incidence of deoxynivalenol was 20%, while other studies show an incidence of 40% for maize flakes and 42.9% for maize flour in Serbia, and 100% for pastry products in Brazil [32,38]. The maximum value identified for deoxynivalenol in this maize study is 152.2 μg/kg, well below the maximum values identified in other studies, while it is 9843.3 μg/kg in China and 5322.4 μg/kg in Belgium [34,37]. In products derived from maize, deoxynivalenol had a maximum of 63.99 μg/kg; other studies indicate a maximum of 931 μg/kg in Serbia and 1720 μg/kg in Brazil [32,38].

In the tested samples of maize, the incidence for zearalenone was 8%. In previous studies on maize, zearalenone had an incidence of 0% in Serbia [45], 13.6% and 94% in China [48], 33.34% in Egypt [47], 40.7%–64.8% in Belgium [34], 41% in Hungary [33] and more than 90% in Germany and Brazil [35,46]. The average value of zearalenone in the Romanian sample was 3.44 μg/kg. Other studies have identified lower average values, such as 2.39 μg/kg or higher in Egypt and 175.5 μg/kg in Belgium [34,47]. The highest zearalenone content in a tested sample was 18.565 μg/kg, while other studies indicate maximum values of 3613 μg/kg [35].

For fumonisins, the incidence in this study was 70% for maize and 25% for products derived from maize. In previous studies, fumonisin incidences ranged from 2.5% in Belgium to 100% in Serbia, Italy, Spain and Canada [34,45,58–60]. The average fumonisin value in the tested samples of maize was 154.55 μg/kg. Other studies have identified an average for fumonisins from 1.5 μg/kg to 10,900 μg/kg [34,58]. For the analyzed products derived from maize, the average value of fumonisins was 37.68 μg/kg. Other studies have identified an average of fumonisins between 14.2 μg/kg and 104.1 μg/kg in corn flakes [59,61].

5. Conclusions

In the present research, regarding contamination with deoxynivalenol, zearalenone and fumonisins in maize and local maize-derived foods, the levels of the three mycotoxins investigated have always been within legal limits. From the three mycotoxins investigated, fumonisins were those with the highest presence, contaminating 70% of the corn samples analyzed. Deoxynivalenol contaminated 25% of the corn samples investigated and zearalenone had the lowest percentage (8%), with the risk of contamination being minimal. Regarding the samples of maize-based foods investigated, fumonisins were detected in 25% of the samples, deoxynivalenol was detected in 20% of the samples and no sample was contaminated with zearalenone.

The investigations carried out in this study, and the comparisons with previously published studies, provide information on the contamination of maize-based foods in Romania by three of the most important mycotoxins, as well as information on their overall distribution. The low percentage of foodstuffs derived from maize contaminated by mycotoxins identified in this study shows that good manufacturing practices are essential for safe food production.

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