

Co-occurrence of aflatoxins and fumonisins in corn food from Serbia in the 2012 production year

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Abstract

Climate conditions in Serbia in the 2012 production year were favorable for fungal growth and mycotoxins production. The presence of fumonisins and aflatoxins in diverse corn-based food was examined using ELISA methods. The applied methods are rapid; demonstrate satisfactory repeatability and recovery rates 99% and 101% for total fumonisins and aflatoxins, respectively. Analysis of samples from 2012 revealed fumonisin contamination in all investigated samples, whereas aflatoxin contamination was detected in 55% of samples. The content of fumonisins did not exceed the maximum permitted level prescribed by the relevant Serbian, i.e., EU Regulation, whereas 3 samples were declared unacceptable due to increased aflatoxin content. High linear correlation coefficient ($r = 0.947$; $p < 0.005$) between fumonisin and aflatoxin contents in maize products was obtained. The results of the analysis of food for human nutrition strongly suggest the need for such monitoring and control.

Keywords: Aflatoxins, fumonisins, cereal food, ELISA

1. Introduction

Mycotoxins are secondary metabolites naturally produced by fungi. They are toxic substances when present in food and feed. Mycotoxins are frequently found as contaminants in cereals worldwide. Cereals can be contaminated in the field, during the harvest or storage [1].

Aflatoxins are mycotoxins evaluated by the International Agency for Research on Cancer (IARC) monograph [2] as carcinogenic to humans (Group 1). Aflatoxins are known to be genotoxic and carcinogenic, with immunosuppressive properties [3,4]. Evaluation by the IARC has classified *Fusarium moniliforme* toxins as potentially carcinogenic to humans (Group 2B carcinogens) [5], since the presence of fumonisin

B₁ in corn was statistically related with esophageal cancer in patients in South Africa [6], China [7], U.S.A. [8], Italy [9], Kenya [10], Brazil [11] and Iran [12].

Cereals represent the most important source of food in human nutrition. Humans living in regions where maize is a dietary staple are at high risk of exposure to both groups of mycotoxins - aflatoxins and fumonisins [13]. European Union has set down official controls of mycotoxins in cereals and other foodstuffs in Commission Regulation (EC) No 401/2006 [14] that was amended with Commission regulation (EU) No 519/2014 [15]. Legislation limits for aflatoxins are given in Commission Regulation No 165/2010 [16] (Table 1), and for fumonisins in Commission Regulation No 1126/2007 [17] (Table 2).

Table 1. Legislation limits for aflatoxins in maize-based food in the EU and Serbia [16,18,19]

Sample Type	EU and Serbia maximum levels (µg/kg)	
	Aflatoxin B ₁	Sum of aflatoxins B ₁ , B ₂ , G ₁ and G ₂
All cereals and all products derived from cereals, including processed cereal products, with the exception of:	2.0	4.0
Maize and rice to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs	5.0	10.0
Processed cereal-based foods and baby foods for infants and young children	0.1	–
Dietary foods for special medicinal purposes intended specifically for infants	0.1	–

Table 2. Legislation limits for fumonisins in maize-based food in the EU and Serbia [17–19]

Sample Type	EU and Serbia maximum levels (µg/kg)
	Sum of fumonisins B ₁ and B ₂
Unprocessed maize, with the exception of unprocessed maize intended to be processed by wet milling	4000
Maize and maize-based foods intended for direct human consumption	1000
Maize-based breakfast cereals and maize-based snacks	800
Processed maize-based foods and baby foods for infants and young children	200
Milling fractions of maize with particle size > 500 µm	1400
Milling fractions of maize with particle size ≤ 500 µm	2000

In Serbia, the prescribed limits are harmonized with the aforementioned European Regulations [18,19].

symptoms.

Climatic conditions are well-established factors contributing to fungal growth and thus the development of mycotoxins in maize [20]. According to the report of the Republic Hydrometeorological Service of Serbia [21], 2012-production year was characterized by numerous hydrometeorological extremes ranging from heat and cold waves to severe and prolonged drought. As a consequence, dramatically accelerated ripening of the majority of crops has occurred.

Poorer qualitative and quantitative crop yield can be attributed to both unfavorable combination of

temperature and humidity conditions in the periods of the year coinciding with the most critical stages of plant development and inadequate implementation of appropriate agro-technical measures. Such conditions favored the occurrence of *Fusarium* infection and increase in pest population. Weather conditions negatively affected the tasseling, silk production and fertilization of corn. The maize was ready for harvesting as early as at the end of August. This overview of climatic conditions strongly indicates that 2012 production year was characterized by pronounced climatic changes, which were unfavorable for maize production.

Having in mind unusual climatic condition in 2012 and aflatoxin contamination of maize in Serbia, the analysis of maize-based products for human nutrition was performed during spring 2013.

2. Materials and Method

The products, which are most commonly used for human consumption, were selected for the analysis. The samples of “traditional, home-made, healthy food” (samples No 1 – 4) were obtained from a store on Zlatibor mountain (samples 1 and 2) and from the market in Novi Sad (samples 3 and 4). The rest of the samples were obtained from a supermarket in Novi Sad (samples No 5 – 9) (Table 3).

The presence of total aflatoxins and fumonisins (FB) in different corn foods were analyzed by enzyme-linked immunosorbent assay methods, using Ridascreen®FAST Aflatoxin (Art. No. R5202) [22] and Ridascreen® Fumonisin (Art. No. R3401) [23] Ridascreen®Fast test kits (R-Biopharm, Germany). The color intensity is measured photometrically at 450 nm (Multiskan FC, Thermo Scientific, China) and is inversely proportional to the mycotoxin concentration in the sample. According to the manufacturer’s description, the detection limits were 1.75 µg/kg (ppb) for aflatoxins, and 25 µg/kg for fumonisins. The analytical quality of the ELISA method was assured by the use of certified reference materials: TR-A100, lot #A-S-267, TR-F100, lot #F-C-439 and TR-Z100, lot #Z-C-320 (Trilogy Analytical

Laboratory, Washington, USA). Recovery was 101% for aflatoxins and 99% for fumonisins.

Special software, the Rida®Soft Win (Art. No. Z9999, R-Biopharm, Germany) was used for the evaluation of enzyme immunoassays.

3. Results and Discussion

The results of the determination of aflatoxins and fumonisins in corn food samples are presented in Table 3, without correction for recovery.

As it can be seen from the presented results, fumonisin was detected in all samples; however, its content did not exceed maximum permitted levels prescribed by relevant Serbian and EU legislation in none of the samples [17,19]. Despite the relatively small number of investigated samples, the results suggested higher contamination rate, i.e., higher fumonisin contents (average concentration 560 µg/kg) in cornmeal as compared to the corn grits (average concentration 105 µg/kg). Aflatoxins were detected in 55% of investigated samples. Taking into consideration the maximum permitted level of total aflatoxins in maize for human nutrition (4 µg/kg), 3 samples were declared inappropriate for human consumption (Table 3).

Table 3. Contents of fumonisins and aflatoxins in corn products for human consumption, harvest 2012

No of sample	Sample type	FB (µg/kg)	Total aflatoxins (µg/kg)
1.	White cornmeal Zlatibor (producer’s name indicated)	1220	19.87
2.	White cornmeal Zlatibor (producer’s name not indicated)	154	1.91
3.	White cornmeal (Novi Sad market, stone-ground)	961	26.25
4.	Yellow cornmeal (Novi Sad market, stone-ground)	145	ND
5.	Yellow cornmeal, brand-specific	319	5.71
6.	Corn grits (gluten free)	119	2.42
7.	Corn grits Bačka	117	ND
8.	Polenta Srem	80	ND
9.	Corn grits brand-specific	105	ND

ND - not detected

It is to be emphasized that two of three inappropriate samples originated from homemade products. Moreover, the problem of potential synergism of two toxins is also to be highlighted.

Examination of potential correlation between fumonisin and aflatoxin contents in maize products (Figure 1) revealed high linear correlation coefficient ($r = 0.947$; $p < 0.005$).

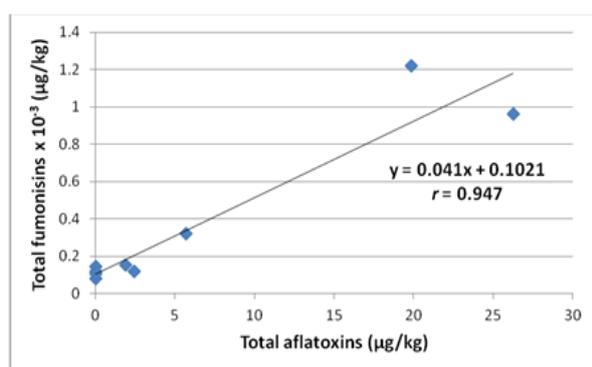


Figure 1. Correlation between fumonisin and aflatoxins contents in maize products for human consumption

The correlation between fumonisin and aflatoxin content in corn products is particularly interesting, since correlation between the content of these toxins in raw corn 2012-harvest could not be confirmed [24]. The obtained results could be explained by different toxin concentrations in different corn-grain parts, which most probably become more prominent during maize processing and production of cornmeal and grits.

In contrast to these results, in all corn food samples from 2010-harvest [25], aflatoxin content was below the detection limit. In four of five analyzed samples of corn-based food, fumonisin content was above the detection limit, yet below the maximum permitted levels for human nutrition. The obtained results were also compared with those obtained in Serbia by other authors. The detections of FB₁ in the cereal-based baby food samples from the organic production were in the range from 331 to 565 µg/kg and FB₂ from 75 to 151 µg/kg. In the conventional production, they were more frequent but lower, i.e., FB₁ and FB₂ ranged from 35 to 86 µg/kg, i.e., from 10 to 85 µg/kg, respectively [26].

Namely, even if fumonisin and aflatoxin contents were below the maximum permitted levels in two samples (No 2 and 6) the fact that both toxins were detected in each sample cannot be neglected.

In spite of the numerous researches addressing the presence of these toxins in maize [27–31], there are not many surveys on co-occurrence of fumonisin and aflatoxins in human foods. The investigation of the presence of mycotoxins in cereals for human consumption conducted in Slovenia in the period 2008-2012 revealed the following most prevalent mycotoxins combinations in corn products: fumonisin – deoxynivalenol and fumonisin – deoxynivalenol – zearalenone. Aflatoxins were not detected in neither of 69 examined samples, whereas fumonisins and zearalenone were confirmed in 9 and 6 of 34 investigated samples, respectively [32]. The examination of cookies and biscuits for the presence of fumonisin and aflatoxin conducted in Iran revealed contamination with both toxins, yet with contents below the maximum permitted levels [33]. The testing of maize-based products for human nutrition in Argentina demonstrated high rate (95%) of fumonisin positive samples, contrary to the results obtained for aflatoxin, zearalenone and DON, which could not be detected [34].

4. Conclusion

Based on the results of the analysis of 2012-harvest corn products we cannot declare the cereal based foods available on Serbian market safe, while the percentage of positive samples imposes the need of more frequent and regular analysis. The results strongly suggest the failure of comprehensive in-depth control in both production process and marketing of those products, even though the unfavorable climatic conditions and apparent contamination of raw corn were indicative enough. Contamination of food samples from regular supermarkets is particularly alarming, whereas contamination of homemade products suggests that they are not necessarily healthier and of better quality, which is commonly expected. Unsafe and inappropriate maize-based products particularly affect the population with celiac disease. They are major consumers of maize-based products, thus, they can face the problem of increased intake of potential corn contaminants such as fumonisins.

This research encompassed the most commonly used maize-based products characteristic for the territory of Serbia. Of course, the dietary habits and food culture change over time with the development of cereal processing technology and a wide variety of maize-based products is available in Serbian market. Increase in number of diverse cereal-based foods imposes new requirements and need for developing and validating novel rapid analytical methods. Namely, having in mind the problems of extraction of bound and hidden fumonisin in processed maize [35], commercial ELISA methods will need further improvement in view of more effective toxin extraction from such complex matrices.

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Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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