CONTAMINATIONS OF RAW MATERIALS AND FOOD PRODUCTS WITH MYCOTOXINS IN SERBIA

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Abstract: *Mycotoxins are secondary fungal metabolites with structural and toxicological properties that induce a variety of toxic and carcinogenic effect when food contaminated with these compounds is ingested. The occurrence of mycotoxins in agricultural commodities depends on the conditions under which a particular crop is grown, harvested or stored. Mycotoxins are stable under most food processing conditions and therefore, persist to the final products. It is, therefore, impossible to eliminate them once the foodstuffs are contaminated. The best protection against mycotoxins is monitoring their presence in feeds and foods. The aim of this work was to determine the content of aflatoxins and ochratoxin A levels in samples of ground paprika and condiments; and content of aflatoxins, ochratoxin A and zearalenone in samples of unprocessed maize, maize based breakfast cereals, maize flour and cereal flour, with regards to maximum tolerable limits in Serbia and European Union. Samples were analyzed by the ELISA (enzyme-linked immunosorbent assay) method. The predominant mycotoxins was zearalenone, who was detected in 37,8% analyzed samples. Aflatoxins were detected in 25.2% of the samples and ochratoxina A in 24.4% of the samples.*

Key words: *Medicinal Aflatoxins, Ochratoxin A, Zearalenone, ELISA*

INTRODUCTION

Mycotoxins are secondary metabolites produced by fungi which contaminate a large variety of foods, with toxic effects on animals and humans (Ghali et al., 2008). Mycotoxins can contaminate a variety of feed and food consumed by animals and humans; essentially cereals, but also fruits, grains, forages, and other products. Plants may be contaminated by mycotoxins in two ways: fungi growing as pathogens on plants or growing saprophytically on stored plants (Glenn, 2007). Most mycotoxins in feed and food are produced by three genera of fungi: *Aspergillus*, *Penicillium* and *Fusarium* (CAST, 2003). Due to their toxic properties and high stability to heat treatment, the presence of mycotoxins in the food chain is potentially hazar-

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dous to the health of both humans and animals (Corrier et al., 1991).

Aflatoxins (AFs) B_1, B_2, G_1 i G_2 are natural substituted coumarones produced by *Aspergillus flavus*, *Aspergillus nomius* and *Aspergillus parasiticus*, which can be found on foodstuffs supporting fungal growth, such as cereals, dried fruits, oil seeds, spices, and pulses (Leitao, 1988). Aflatoxins have immunotoxic, mutagenic and carcinogenic effects, and they were classified as group 1 carcinogenes by the International Agency for research of Cancer (IARC, 1993). Aflatoxin B_1 (Fig. 1, A) is the most frequently encountered of the group and the most toxic (Ghali et al., 2008). Aflatoxins have been extensively documented as food contaminants in Mediterranean region (Zinedine et al., 2006). Ochratoxins are secondary metabolites of Aspergillus and Penicillium strains, found on cereals, coffee and bread, as well as on all kinds of food commodities of animal origin in many countries. The most frequents is Ochratoxin A (Fig 1, B), which is also the most toxic (Speijers, 1993). Ochratoxin A (OTA) is a mycotoxin produced by *Penicillium verrucosum*, *Aspergillus ochraceus*, and other related species (MYC-CONF, 1999). OTA is responsible for nephrotoxic, immunosupresive and carcinogen (group 2B) (IARC,1993). OTA can be found in corn, barley, green coffee and various dried fruits, and it is widespread on the region of Serbia and the rest of Balkans. It might be present in conjuction with aflatoxins.

Zearalenone (ZEA) (Fig 1, C) is produced mainly by the mold *Fusarium graminearum* and *Fusarium sprotrichoides,* in the field and during storage, principally in wheat and maize but also in sorghum, barley and compounded feeds. ZEA is classified as an estrogenic mycotoxin because it frequently causes estrogenic responses in animals. ZEA is classified by IARC under group 3 carcinogen (IARC, 1999).

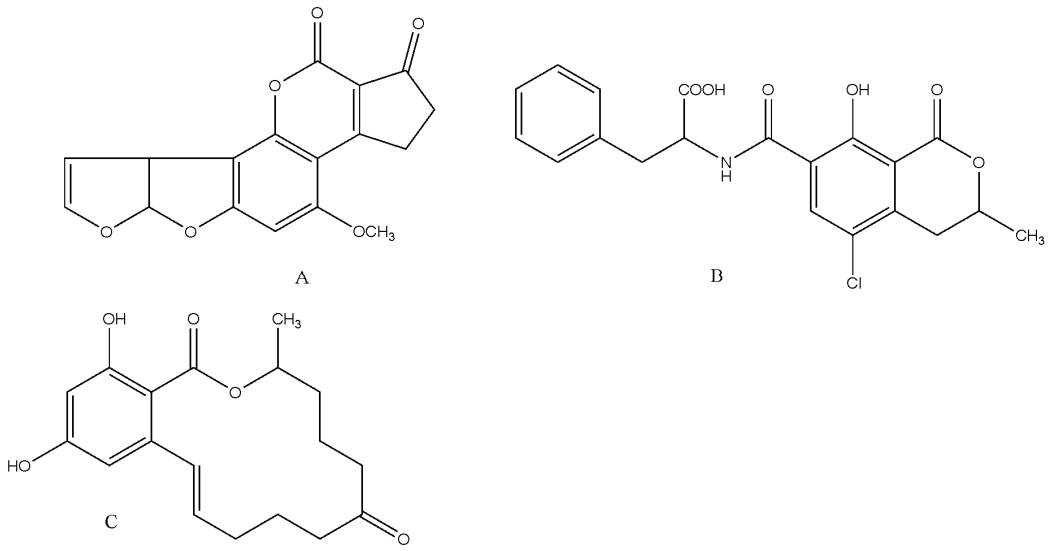

Figure 1. Structures of some mycotoxins: A- aflatoxin B_1, B-ochratoxin A, C-zearalenone

The best protection against mycotoxins is monitoring their presence in feeds and foods. Since mycotoxins can cause serious problems with human healths, the maximum tolerated levels of mycotoxins in food products have been established in many countries. Allowed limits of mycotoxins in food in Serbia are determined by regulations of Serbia („Sl. list SRJ“, br. 5/92,11/92, 32/02). Allowed limits of mycotoxins in food on teritory of European Union are regulated by regulation of European Union (EC 1881/-2006). Maximum allowed limits for aflatoxins and ochratoxin A in Serbia and EU for many food products are similar or somewhat different; and regarding zearalenone, regulations of Serbia

and EU are very different, with allowed being much lower in Serbia.

MATERIALS AND METHODS

A total of 123 samples were collected during the 2008. from different shops in Serbia. The amounts of aflatoxins and ochratoxin A were determined in 18 samples of ground paprika and 23 samples of condiments; and amounts of aflatoxins, ochratoxin A and zearalenone in 43 samples of unprocessed maize, 9 samples of maize based breakfast cereals, 17 samples of maize flour and 13 samples of cereal flour. Samples were analyzed by the ELISA (enzyme-linked immunosorbent assay) method. Screening method for analysis was done using Neogen Veratox® testing kits with limits of detection of 1 µg/kg (ppb) for aflatoxins, 2 µg/kg (ppb) for ochratoxin A and 25 µg/kg (ppb) for zearalenone. Each sample was extracted by confinable solvents system; methanol-distilled wather (70/30) for AFs and ZEA, and methanol-distilled wather (50/50) for OTA. The optical densities of the controls form the standard curve, and the sample optical densities are plotted against the curve to calculate the exact concentration of mycotoxin (Veratox®, Neogen).

RESULTS AND DISCUSSION

Eighteen samples of ground paprika have been analyzed. Ground paprika can be contaminated with mycotoxins, such as aflatoxins, ochratoxins and other mycotoxins. These mycotoxins very often contaminate parika in countries with warm and humid climates and with inappropriate storage conditions. The results of test on aflatoxins and ochratoxin A in 18 samples, as well as reference values for Serbia and EU are shown in Table 1.

Table 1.
Result of analyzed ground paprika for the presence of aflatoxins and ochratoxin A

Type of mycotoxin	Number of samples	Mycotoxin content (µg/kg)	Reference values (µg/kg)	
			Serbia	EU
Aflatoxin	1	< 1	30	10
	12	1-5		
	5	5-10		
	/	> 10		
Ochratoxin	9	< 2	10	/
	8	2-4		
	1	>4		

Presence of aflatoxins was detected in seventeen samples of ground paprika. In twelve samples were detected aflatoxins in the amount of 1 to 5 µg/kg, and in five samples of 5 to 10 µg/kg. The values determined are below the limits of both Serbia and European Union reference values. Ochratoxin A is detected in nine samples of ground paprika, but its content is below the maximum tolerated values.

Twenty three samples of condiments have been analyzed. Condiments can be contaminated with molds and mycotoxins because they are mixture of a number of different ingredients. Aflatoxins were detected in only five samples of condiments in amount of 1 to 2 (µg/kg). Ochratoxin A were detected in eleven samples in amount of 2 to 6 (µg/kg), and in five samples in amount of 6 to 10 (µg/kg). In analyzed samples of condiments content of aflatoxins and ochratoxins are below the limits of both Serbia and European Union reference values (Table 2).

Table 2.
Result of analyzed condiments for the presence of aflatoxins and ochratoxin A

Type of mycotoxin	Number of samples	Mycotoxin content (µg/kg)	Reference values (µg/kg)	
			Serbia	EU
Aflatoxin	18	< 1	30	10
	5	1-2		
	/	> 2		
Ochratoxin	7	< 2	10	/
	11	2-6		
	5	6-10		
	/	> 10		

Thirty eight samples of unprocessed maize have been analyzed (Table 3). Aflatoxins have been detected in five samples, and ochratoxins in two samples. Content of aflatoxins and ochratoxins in analyzed samples of unprocessed maize are below the limits of both Serbia and European Union reference values. In thirty eight samples of unprocessed maize, zearalenone have been detected in eighteen samples. The Serbian limit for ZEA in unprocessed maize is 1 µg/kg, and the Europian regulatory limit for ZEA is 200 µg/kg. In seven samples zearalenone were detected in the amount of 25 to 75 µg/kg, and in three samples of 75 to 200 µg/kg. In eight samples zearalenone were detected in the amount higher than 200 µg/kg, and this samples are not in accordance with the European regulatory. All tested samples are not in accordance with the Regulation of Serbia. Maize is a very often contaminated with zearalenone. Maize can be contaminated with molds and zearalenone in the field, or during the harvest or stored. Unprocessed maize who are contaminated with high content of zearalenone (>200 µg/kg), can not be used for nutrition human and animal, or production different kind of food or feed.

In nine samples of maize based breakfast cereals aflatoxins, ochratoxin A and zearalenone have been detected in only one sample, and that values are in accordance with Serbia and European Union regulatory (Table 3).

Of the seventeen samples of maize flour tested, in six samples zearalenone have been detected. In three samples zearalenone were detected in the amount of 25 to 75 µg/kg, and in three samples of 75 to 200 µg/kg. In only one sample zearalenone were detected in the amount higher than 200 µg/kg, and these samples are not in accordance with the European regulatory. Aflatoxins and ochratoxin A have been detected in two samples. Content of aflatoxins and ochratoxins in analyzed samples of maize flour are below the limits of both Serbia and European Union reference values.

Thirteen samples of cereal flour have been analyzed (Table 4). In eight samples zearalenone have not been detected, and in three samples zearalenone were detected in amount of 25 to 75 µg/kg. In one sample amount of zearalenone is higher than reference values in European Union regulatory. Due to maximum tolerable limits of zearalenone in flour in Serbia is 1 µg/kg, concentrations in all analyzed samples of flour are considerably higher than the reference values in Serbia. Aflatoxins have been detected in one sample of cereal flour.

Table 3.
Result of analyzed unprocessed maize and maize basedbreakfast cereals for the presence of zearalenone

Sample type	Number of samples	Mycotoxin content(µg/kg)	Reference values (µg/kg)	
			Serbia	EU
Unprocessed maize	25	< 25	1	200
	7	25-75		
	3	75-200		
	8	> 200		
Maize based breakfast cereals	8	< 25	1	50
	1	25-50		
	/	>50		

Table 4.
Result of analyzed flour and cereal flour for the presence of zearalenone

Sample type	Number of samples	Mycotoxin content(µg/kg)	Reference values (µg/kg)	
			Serbia	EU
Maize flour	10	< 25	1	200
	3	25-75		
	3	75-200		
	1	> 200		
Cereal flour	8	< 25	1	75
	3	25-75		
	2	>75		

CONCLUSION

Results showed that the content of aflatoxins and ochratoxin A in all investigated samples are in accordance with the Serbian and European standard. Of the 82 samples tested on zearalenone, 11 samples are not in accordance with EU standard and all investigated samples are not in accordance with Serbian standards. This shows that regulations in Serbia should be changed and corrected in accordance to widely accepted regulations within European Union.

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