

AFLATOXINS AS FOOD CONTAMINANTS

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Abstract: Mycotoxins are nowadays one of the most known and most frequent chemical food contaminants. Among the listed mycotoxins, aflatoxins are the most important group of mycotoxins in terms of impact on human and animal health. Of the known mycotoxins, aflatoxin B1 is considered the most toxic. The main sources of aflatoxins are cereals, oilseeds, nuts, dried fruit, and spices. The most commonly used screening method for the detection of mycotoxins is the immunoenzyme (ELISA) method. The purpose of this paper is to show the types of foodstuffs and the results of sample analyses of aflatoxin B1 in these foodstuffs carried out at the Institute of Public Health of the Republic of Srpska - Regional Center Doboj in the period from 2011 to 2017, and to propose measures for improvement of supervision in this area, i.e. to recommend certain measures in order to prevent the occurrence of aflatoxins in foodstuffs.

Key words: aflatoxins, food, analysis.

INTRODUCTION

Mycotoxins are nowadays one of the most known and most frequent chemical food contaminants (Kos, 2015). They are produced as secondary products of mold metabolism, where they can contaminate a large number of different agricultural and food products, as well as animal feed. About 400 mycotoxins have been discovered to date, but the greatest attention is given to those most commonly occurring as contaminants of food and feed, such as: ochratoxins, zearalenone, fumonisins, trichothecenes, patulin (Marasas et al., 2008). Among the listed mycotoxins, aflatoxins are the most important group of mycotoxins in terms of impact on human and animal health. Numerous studies have found that certain mycotoxins have neurotoxic, hepatotoxic, genotoxic, carcinogenic, immunosuppressive, and estrogenic effects (Domijan and Peraica, 2010; Kos, 2015). Human and animal diseases caused by the effects of mycotoxins are commonly called mycotoxicoses.

Like other mycotoxins, aflatoxins can contaminate various agricultural and food products, as well as animal feed, and thus lead to multiple negative effects on human and animal health, and at the same time their occurrence may cause major economic losses (Greppy et al., 2002). The main sources of aflatoxins are cereals, oilseeds, nuts, dried fruit, and spices (Turner et al., 2009; Salem and Ahmed, 2010). If there are appropriate physical, chemical and biological conditions, *Aspergillus* species will grow, develop and reproduce, and with the presence of a genetic predisposition, mold will also synthesize aflatoxins (Payne, 1992; Hesselstine, 1979).

According to various authors, between 13 and 20 different aflatoxins have been discovered to date. Of the known mycotoxins, aflatoxin B1 is considered the most toxic, and according to the criteria of the United Nations Economic Commission for Europe (GHS), it belongs to substances with acute toxic effects of the first category in the case of oral and percutaneous exposure. Due to its cumulative effect in the organs, aflatoxin B1 is characterized as a carcinogenic substance for humans and it is classified in group 1A according to the International Agency for Research on Cancer (IARC, 2002).

The implementation of systematic control of aflatoxin occurrence is based on the application of analytical methods that should meet the criteria of contemporary analytical practice in terms of sensitivity

and accuracy, and at the same time it requires simple and quick execution of the analysis at an affordable price (Kralj Cigić and Prosen, 2009). Different screening and confirmatory analytical methods are used for the purpose of detecting aflatoxins. The most commonly used screening method for the detection of mycotoxins is the immunoenzyme (ELISA) method (Perši, 2012). Literature data indicate that the ELISA method has a number of advantages over other analytical methods as far as the detection of aflatoxins is concerned. These advantages primarily relate to the speed of analysis performance, the ability to analyze a large number of samples in a short period, high specificity, simplicity, low cost, and the use of harmless reagents (Pestka, 1994; Zheng et al., 2005; Goryacheva et al., 2007; Ayejuyo et al., 2011).

Since 2011, in the Laboratory of sanitary chemistry of the Institute of Public Health of the Republic of Srpska - Regional Center Doboj, samples have been analyzed by the ELISA method for the identification of aflatoxin B1 and total aflatoxins. Since 2015, this method has been accredited according to BAS EN ISO/IEC 17 025:2006.

The purpose of this paper is to show the types of foodstuffs and the results of sample analyses of aflatoxin B1 in these foodstuffs carried out at the Institute of Public Health of the Republic of Srpska - Regional Center Doboj in the period from 2011 to 2017, and to propose measures for improvement of supervision in this area, i.e. to recommend certain measures in order to prevent the occurrence of aflatoxins in foodstuffs.

MATERIAL AND METHODS

This study was conducted as a retrospective study on 1,333 samples of various foodstuffs of foreign trade in the Republic of Srpska (Bosnia and Herzegovina) in the period 2011-2017, that were submitted to the Institute of Public Health of the Republic of Srpska - Regional Center Doboj. The Central protocol of the laboratory within the Institute of Public Health of the Republic of Srpska - Regional Center Doboj was used as a data source. The samples were divided into 4 groups: cereals and cereal products, fruit and fruit products, vegetables and vegetable products, and other types of foodstuffs. The content of aflatoxin B1 was determined by the ELISA method using the kits made by Proxima, a company from the Netherlands (according to the user manual). The analysis that determined whether the samples were safe for our health or not was done in accordance with the relevant food safety regulations valid during the research period (Rulebook 2009, Rulebook 2012, Rulebook 2014, Rulebook 2016). The results of the analyses are presented in the form of tables. Descriptive statistics were used in data processing.

RESULTS

In the period from 2011 to 2017, a total of 1,333 samples were analyzed for the identification of aflatoxin B1 in the Laboratory of sanitary chemistry of the Institute of Public Health of the Republic of Srpska - Regional Center Doboj (Table 1, Chart 1). The largest number of analyzed samples was recorded in 2016 (314), and the lowest in 2012, 35 samples. From all the above mentioned samples, only in 5 cases, or 0.37%, all originating from 2013, there was a positive result for the B1.

Table 1: Number of analyses of food samples for aflatoxin B1 in the period 2011-2017

Year	2011	2012	2013	2014	2015	2016	2017	TOTAL
Total	135	35	266	291	173	314	119	1333
Contaminated	0	0	5	0	0	0	0	5

The structure of the samples is shown in Table 2, which clearly shows that the group of foodstuffs - cereals and cereal products - was most often subject to analysis of aflatoxin B1 (77.2%), followed by foods

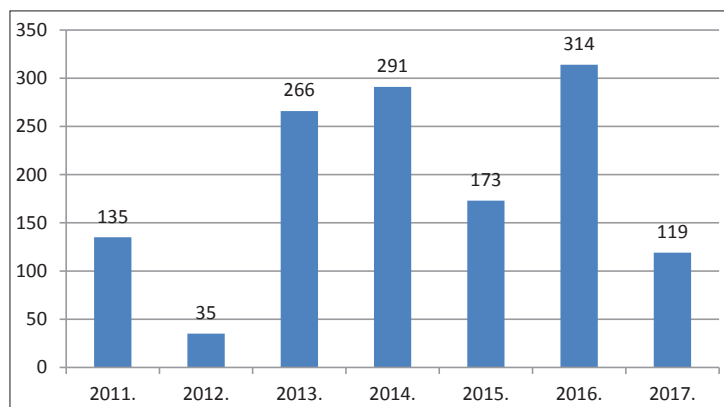


Chart 1: Graphic presentation of the number of food samples analyzed in different years

from the group of fruit and fruit products (14.4%), while only 2.5% of the analyses referred to vegetables and vegetable products.

Table 2: Number and percentage of samples analyzed for aflatoxin B1 with respect to the group of foodstuffs

Cereals and cereal products		Fruit and fruit products		Vegetables and vegetable products		Other types of foodstuffs		TOTAL	
Number	%	Number	%	Number	%	Number	%	Number	%
1030	77,2	193	14,4	35	2,5	79	5,9	1333	100,00

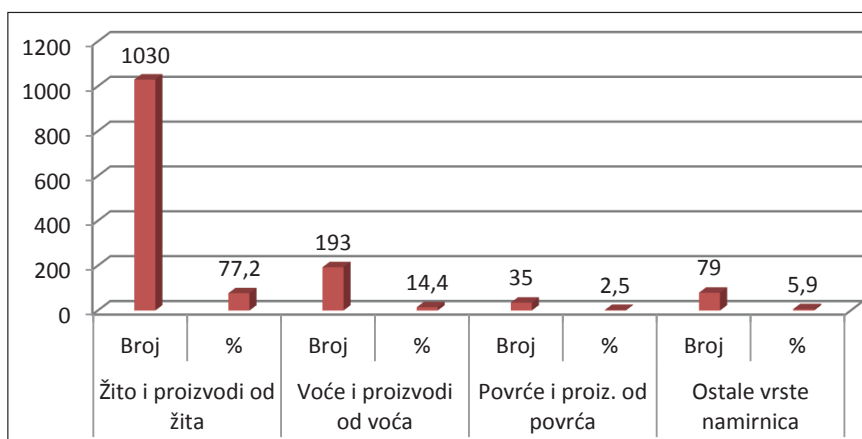


Chart 2: Graphic presentation of the analyses of aflatoxin B1 with respect to the groups of foodstuffs

Table 3 gives an overview of the analyses of samples of cereals and cereal products in the observed period. We have already pointed out that with a total of 1,030 analyzed samples, this group of foodstuffs has most often been subject to the analysis of aflatoxin B1. Thus, the already mentioned positive results for aflatoxin B1, only 5 samples, or 0.48%, just refer to this group of foodstuffs. If we observe the analysis of samples of certain subgroups within the group of cereals and cereal products, it can be noted that 4 positive results relate to the samples of grain, and one to mill products.

Table 3: Overview of analyses of samples of cereals and cereal products for aflatoxin B1 in the period 2011-2017

Year		2011	2012	2013	2014	2015	2016	2017	TOTAL
Cereals and cereal products	Total	129	20	206	221	134	228	92	1030
	Contaminated	0	0	5	0	0	0	0	5
Grains	Total	110	19	90	128	98	105	16	566
	Contaminated	0	0	4	0	0	0	0	4
Mill products	Total	19	0	109	93	36	123	76	456
	Contaminated	0	0	1	0	0	0	0	1
Pasta and related products	Total	0	1	2	0	0	0	0	3
	Contaminated	0	0	0	0	0	0	0	0

Table 4 and Table 5 provide an overview of the analyses of samples of fruit and fruit products as well as vegetables and vegetable products. It is noticeable that the samples of fruit and fruit products were more than five times more frequently analyzed than the samples that were related to vegetables and vegetable products. It is significant that neither of these two groups contained samples positive to aflatoxin B1.

Table 4: Overview of analyses of samples of fruit and fruit products for aflatoxin B1 in the period 2011-2017

Year		2011	2012	2013	2014	2015	2016	2017	TOTAL
Fruit and fruit products	Total	2	0	39	44	32	51	25	193
	Contaminated	0	0	0	0	0	0	0	0
Nuts	Total	2	0	29	32	29	45	24	161
Dried fruit	Total	0	0	5	3	2	5	1	16
Other products	Total	0	0	5	9	1	1	0	16

In the group of fruit and fruit products, the dominance of analyses related to samples of nuts is noticeable.

Table 5: Overview of analyses of samples of vegetables and vegetable products for aflatoxin B1 in the period 2011-2017

Year		2011	2012	2013	2014	2015	2016	2017	TOTAL
Vegetables and vegetable products	Total	0	0	4	4	2	24	1	35
	Contaminated	0	0	0	0	0	0	0	0

Table 6 gives an overview of the samples that are not classified into any of the aforementioned food groups: spices were analyzed in 5 cases, a total of 19 samples of coffee, 34 samples of cocoa and candy products, 15 samples of biscuits and related products, and 5 other samples.

Table 6: Overview of analyses of samples of other food types for aflatoxin B1 in the period 2011-2017

Year		2011	2012	2013	2014	2015	2016	2017	TOTAL
Spices	Total	1	0	0	0	0	4	0	5
	Contaminated	0	0	0	0	0	0	0	0
Coffee	Total	2	0	1	11	5	0	0	19
	Contaminated	0	0	0	0	0	0	0	0
Cocoa and candy products	Total	0	11	11	5	0	7	0	34
	Contaminated	0	0	0	0	0	0	0	0

Biscuits and related products	Total	0	4	7	4	0	0	0	15
	Contaminated	0	0	0	0	0	0	0	0
Other products	Total	1	0	3	3	0	0	1	7
	Contaminated	0	0	0	0	0	0	0	0

DISCUSSION

The main source of mycotoxins in humans are cereals, cereal-based products, and nuts. The Food and Agriculture Organization (FAO) estimates that globally 25% of crops intended for human or animal consumption are contaminated with mycotoxins (Pleadin et al., 2014). And aflatoxins pose the greatest risk in the food chain (Scholthof, 2003). In accordance with the previous facts, the analysis of aflatoxin B1 in the Laboratory of sanitary chemistry of the Institute of Public Health of the Republic of Srpska - RC Doboj mostly covered the samples of cereals and cereal products (77.2%) and samples of fruit and fruit products (14.4%), which basically were samples of different nuts. It is important to emphasize that most of the analyzed samples were foodstuffs imported through the main border crossings for imports of cereals and cereal products (in Gradiška, Šamac, Rača, and Karakaj - Zvornik). For example, in 2016, 398,756 tons of wheat and 303,078 tons of corn were imported into Bosnia and Herzegovina. A somewhat smaller number of analyses were recorded in 2015, and especially in 2017, which can be “justified” by the fact that the Laboratory of sanitary chemistry of the Institute of Public Health of the Republic of Srpska in Banja Luka has been involved in the detection of aflatoxins since 2015.

In the Laboratory of sanitary chemistry of the Institute of Public Health of the Republic of Srpska - Regional Center Doboj, no analysis was done of cereals and cereal products that were produced on the fields in the Republic of Srpska, Bosnia and Herzegovina. For illustration purposes, in 2016, 1,178,423 tons of corn and 306,605 tons of wheat were produced in Bosnia and Herzegovina, of which 880,998 tons of corn and 194,311 tons of wheat were produced in the Republic of Srpska. Although tropical climatic conditions are most favorable for the occurrence of aflatoxins in cereals and nuts, changes in climatic conditions in the form of longer periods with high daily minimum temperatures lead to an increased risk of the occurrence of mycotoxins in a moderate climate zone, too (Russell et al., 2010). High average temperature and a long period of drought cause thermal stress in plants, wherein the plants are contaminated by molds of the *Aspergillus* species, especially in the period when corn is flowering and corn silk is getting darker (Marsh and Payne, 1984; Kocić-Tanackov and Dimić, 2013). High temperatures and drought periods also favor the colonization of various insects and lead to cracking and damage of corn grains, further enabling the synthesis of toxins. Precisely these weather conditions, i.e. with the optimal temperature for the development of mold in the range of 25 to 42°C (Santin, 2005), were recorded in 2012 in particular. Thus, as a result of the aforementioned weather conditions in the year 2012, in the samples of corn grown in that year on the fields in Serbia, the aflatoxin B1 values exceeded the prescribed maximum permissible concentrations (Kos et al., 2013; Spirić et al., 2015). We also find papers that report on the contamination of corn and compound feed for cattle with aflatoxin B1 produced in the Republic of Croatia, which was also in 2012, and consequently on the contamination of milk from dairy farms (Pleadin et al., 2014; Bilandžić et al., 2014; Pleadin et al., 2015). Contaminated samples of cereals and cereal products, a total of 5 in the observed period from 2011 to 2017, were registered in 2013, but the samples referred to the cereals produced in 2012. Regarding the number of sample analyses in the “risky” year 2012, contrary to the expected increased controls, only 35 samples were analyzed in 2012 (Table 1), of which there were 20 samples of cereals and cereal products (Table 2), which is evidently the lowest number compared to the years of the observed period of several years.

But if we return to the control of samples of cereals from domestic production, then we have to point out that this part of the control in the Republic of Srpska is performed by other laboratories, first of all, by the Agricultural Institute of the Republic of Srpska - Banja Luka. Unlike the Institute of Public Health of the Republic of Srpska, whose website contains annual reports containing data on laboratory analyses of foodstuffs, on the website of the Agricultural Institute of the Republic of Srpska we can only learn that it performs analyses of foods for mycotoxins, but there are no reports on these analyses. Here, our intention was, basically, to emphasize the necessity of unifying the data on the performed analyses of all types of foods, including milk and dairy products (which were not the subject of this work), and, in particular, that such reports (annual) are available to the expert public, and in a certain form also to the general public (consumers). In 2013, European Food Safety Authority (EFSA) emphasized the need to collect data on the occurrence of aflatoxins in cereals and cereal products and the need to align reporting on established concentrations of these toxins at the level of all European countries (EFSA, 2013).

Other types of foodstuffs, whose samples were analyzed, were also basically imported. Considering the amount of imports of these foodstuffs and the risk of aflatoxin contamination, we believe that the expected participation of these foods in the aflatoxin analyses has been achieved.

Along with the establishment of a system for monitoring the aflatoxins, primarily in cereals and cereal products, it is important to work on curbing the formation of aflatoxins. Namely, there are numerous ways to eliminate already created mycotoxins in foodstuffs and raw materials, but none is completely effective. The level of degradation of mycotoxins depends on the type of foodstuff, the size of the individual particles, the moisture content, the amount of mycotoxins, the location of the toxin, the temperature at which the treatment is performed. The structure of aflatoxin B1, in particular, is destroyed only at temperatures higher than 250°C. This practically means that the prevention of the formation of aflatoxin B1, or the prevention of food contamination by aflatoxin B1, is the most effective way of combating the toxins. This includes hygienic measures in the production, processing, storage, and transport of foodstuffs, as well as avoiding conditions suitable for the development of mold. Practically, this describes a modern production of healthy and quality products based on the “from farm to table” principles, HACCP (Hazard analysis and critical control points) system, i.e. hazard analysis, prevention, control of critical points, and the removal of potential hazards in technological process of production (Asefa et al., 2011).

Twenty years ago, it was pointed to the possibility of reducing aflatoxin contamination in the pre-harvest period, at least in the case of some types of cereals, by using genetic engineering and the development of hybrid varieties resistant to infection by various strains of mold of the *Aspergillus* species (Widstrom, 1996). As a way of reducing the risk of contamination with molds and mycotoxins in the field, a crop rotation is proposed (Jaime-Garcia and Cotty, 2010). Storage conditions after harvest have also been of great importance in the prevention of mycotoxins. Seed sorting and drying are important parameters for preventing subsequent contamination of cereals with mycotoxins (Cotty and Jaime-Garcia, 2007; Birck et al., 2006). When storing cereal grains and nuts, attention must primarily be focused on maintaining water activity at a level lower than that which facilitates the growth of mold (IARC, 2002). There are suggestions in this regard that even propose to collect only those crops whose grain moisture content is approximately 24% (Prandini et al., 2009).

CONCLUSION

- In the period 2011-2017, a total of 1,333 samples of various foodstuffs from imports were analyzed for aflatoxin B1 in the Laboratory of sanitary chemistry of the Institute of Public Health of the Republic of Srpska - Regional Center Dobo.

- When it comes to the type of foodstuffs whose samples were analyzed for the presence of aflatoxin B₁, the most common were cereals and cereal products (77%) and fruit and fruit products (14.4%).
- Only 5 samples were positive for aflatoxin B₁, all related to cereals and cereal products produced during the period when weather conditions were favorable for the development of mold.
- In the years when the production was at a higher risk for the occurrence of mold, it happened that the subject laboratories analyzed a smaller number of samples compared to the production from less “risky” years.
- It is necessary to establish appropriate systematic monitoring for aflatoxins in foodstuffs, both from imports and domestic production, and an annual reporting on the results of the analyses of all types of foodstuffs for mycotoxins, which should be available to experts, but also to the general public (consumers).

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