JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2011, *17*(2), 108-112

Journal of Agroalimentary Processes and Technologies

Incidence of aflatoxins and fumonisins in cereal food from Serbian market

Sandra M. Jakšić[1], Biljana F. Abramović[2], Bojana Z. Prunić[1], Željko A. Mihaljev[1], Milica M. Živkov Baloš[1], Igor M. Jajić[3], Vesna N. Despotović[2], Luka J. Bjelica[2]

[1] *Scientific Veterinary Institute „Novi Sad", Rumenački put 20, 21000 Novi Sad, Serbia, sandra@niv.ns.ac.rs*
[2] *Faculty of Science, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia, biljana.abramovic@dh.uns.ac.rs*
[3] *Faculty of Agriculture, Department of Animal Science, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia, igor.jajic@gmail.com*

Received: 11 May 2011; Accepted: 19 June 2011

Abstract

Cereal food has an important role in traditional Serbian nutrition and is also very attractive in modern nutrition as so-called `healthy food`. Cereals are possible sources of mycotoxins, but there is not enough information about their presence in food produced in Serbia. To investigate the incidence of aflatoxins and fumonisins, different cereal foods were analyzed by enzyme-linked immunosorbent assay methods. The used methods appeared to be rapid and reproducible, with recovery of 94.3% for aflatoxins and 98.0% for total fumonisins. In 33.3% of samples, content of aflatoxins was above the limit of detection, but in all samples it was lower than the maximum allowed by Serbian regulations. In four out of five analyzed corn food samples, content of fumonisins was detectable, but lower than the limit set by the regulations of the European Union. The results showed the necessity of such tests.

Keywords: Aflatoxins, fumonisins, cereal food, ELISA

1. Introduction

Mycotoxins are fungi toxins, frequently found as contaminants in cereals worldwide. In terms of exposure and severity of chronic disease, especially cancer, mycotoxins appear at present to pose a higher risk than anthropogenic contaminants, pesticides and food additives [1].

Aflatoxins are mycotoxins of the highest toxic importance. Aflatoxin B1 is the most common in food and amongst the most potent genotoxic and carcinogenic aflatoxins. It is produced both by Aspergillus flavus and Aspergillus parasiticus. Studies evaluated in the International Agency for Research on Cancer (IARC) monograph [2] have led to the classification of naturally occurring aflatoxins as carcinogenic to humans (Group 1). Since aflatoxins are known to be genotoxic and carcinogenic, exposure through food should be kept as low as possible.

Fumonisin B1 is the most prevalent member of a family of toxins produced by several species of Fusarium molds which occur mainly in corn. Fumonisin B1 contamination of corn has been reported worldwide at mg/kg levels, and our previous research proved the presence of fumonisins in Serbian corn [3]. Evaluation by the IARC has classified Fusarium moniliforme toxins as potentially carcinogenic to humans (Group 2B carcinogens) [4].

In Serbian nutrition, cereals (especially wheat and corn) are common ingredients in some traditional meals.

Corresponding author: *e-mail:* biljana.abramovic@dh.uns.ac.rs

On the other hand, in modern nutrition for younger population, very popular are breakfast cereals. Although maximum levels for some mycotoxins are included in Serbian legislation for cereal-based foods, there is not enough information about the results of mycotoxicological food control. The differences in the European Union (EU) and Serbian regulations are evident from Table 1.

Table 1. Legislation limits for aflatoxins and fumonisins in cereal food in the EU and Serbia [5,6,7]

Sample Type	EU maximum levels (ppb)		Serbia maximum levels (ppb)
	Aflatoxin B_1	Total aflatoxins	Aflatoxins B_1+G_1
Wheat, corn, rice, barley and other cereals	2	4	5
Cereal flour	–	–	3
Processed cereal-based foods and baby foods for infants and young children	0.1	–	–
	Fumonisins B_1+B_2		
Corn and corn-based foods intended for direct human consumption	1000		–
Corn-based breakfast cereals and corn-based snacks	800		–
Baby foods for infants and young children	200		–

The aim of this study was to investigate the incidence of aflatoxins and fumonisins in different cereal foods available at Serbian market.

2. Materials and methods

In this study, 45 samples of cereal food were analyzed for the incidence of mycotoxins. Samples, either original packs or in bulk, were collected from the supermarkets. Out of them, 35 samples originated from Serbia and 10 were from import.

After grinding, homogenization, extraction with methanol (70 or 80%) and extract dilution, contents of total aflatoxins and total fumonisins were determined by the enzyme-linked immunosorbent assay (ELISA) method, using Aflatoxin-B1 EIA (code 5121AFB1p, EuroProxima, The Netherlands) and Ridascreen® Fumonisin test kits (Art. No. R3401, R-Biopharm, Germany). The basis of the tests is the antigen-antibody reaction. The microtiter wells are coated with capture antibodies directed against anti-mycotoxin antibodies. Mycotoxin standards or sample solutions, mycotoxin enzyme conjugate and anti mycotoxin antibodies are added. Free mycotoxin and mycotoxin enzyme conjugate competes for the mycotoxin antibody binding sites (competitive enzyme immune-assay).

At the same time, the anti-mycotoxin antibodies are also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. Substrate/chromogen is added to the wells, the bound enzyme conjugate converts the chromogen to a colored product. The substrate reaction is stopped by the addition of the stop solution (sulfuric acid). The color intensity is measured photometrically at 450 nm (Multiscan MCC/340, Labsystem, Finland) and is inversely proportional to the mycotoxin concentration in the sample. According to the manufacturer´s description [8,9], the detection limits for aflatoxins and fumonisins were 0.3 µg/kg (ppb) and 25 µg/kg, respectively.

Recovery was determined using the samples spiked with an appropriate amount of standard solutions of aflatoxin B1 (0.1 µg/ml) and fumonisin B1 (10 µg/ml).

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

3. Results and Discussion

Before sample analysis, recoveries for both used ELISA tests were determined. Recovery for aflatoxin B_1 determined on barley flakes sample spiked with 3 ppb was 94.3±12.6%, and for fumonisin B_1 for corn flakes sample spiked with 800 ppb 98.0±26.8%.

Results of the determination of aflatoxins in cereal food samples are presented in Table 2, without correction for recovery.

As can be seen, aflatoxins are not detected in baby cereal food. It should be noted that the limit of detection for baby food and rice was significantly higher (0.7 ppb) than for other samples, because of their nature.

These samples imbibe extraction solution and higher volume is needed for extraction. It should be pointed out that the detection limit of the applied ELISA test (0.3 ppb) was not low enough, as the maximum tolerable limit for aflatoxins in European Union is only 0.1 ppb (Table 1) [6].

Although we did not detect aflatoxins in the baby food samples, it would be advisable to examine them for mycotoxin contamination by a more sensitive ELISA (Europroxima, code 5121AFSS1p) or by liquid chromatography method [10,11,12].

Aflatoxins were not detected in the samples of rye, oats and corn. However, barley, soy and mixed breakfast cereals appeared to be contaminated to a significant percentage, i.e. 50, 66.7 and 63.6%, respectively, although the analyzed samples were not numerous. Content of aflatoxins in all samples was below the maximum allowed by Serbian regulation (Table 1), but in one expanded barley sample, aflatoxins content was higher than 4 ppb, mentioned as the upper limit in the EU regulations.

However, the criterion of the appropriateness of these samples depends on whether breakfast cereals are to be considered as a food for younger children. The obtained results were compared with those obtained in Serbia by other authors [13]. In contrast to us, these authors found that none of the 76 examined grain samples was positive for aflatoxins. However, it should be pointed out that they used an ELISA test kit with a higher detection limit (1 ppb). Our results are similar to those obtained in the evaluation of aflatoxins contamination of breakfast cereals from Athens market, where the mean content of 1.42 ppb was found in 56.3% samples [11]. Contrary to this, in Tunisian cereal food, aflatoxins were present in higher concentrations, up to 40.6 ppb [14]. In Canada, 50% breakfast and infant cereals had detectable levels of aflatoxin B_1, with a maximum of 1.0 ppb [12], whereas in Spain, 66% infant cereal samples were positive on total aflatoxins [15].

If the origin of the samples is analyzed (Table 3), it can be seen that bulk samples had good storing conditions in Serbia, since they contained aflatoxins below the detection limit of the ELISA test employed. Imported products were contaminated to a somewhat higher percentage, but the mean content of aflatoxins was lower than in cereals of Serbian producers.

Table 2. Aflatoxins content in cereal food

Sample Type	No. of samples	No. of positive samples	Range of aflatoxins contamination (ppb)	Mean of positive samples (ppb)
Baby food	6	–	–	–
Rice flakes and expanded rice	3	1	2.70	2.70
Barley flakes and expanded barley	6	3	0.364 – 4.08	2.76
Wheat flakes, expanded wheat and grits	6	1	0.538	0.538
Rye flakes	2	–	–	–
Soy flakes	3	2	0.537 – 1.32	0.928
Buckwheat	1	1	0.71	0.71
Oats flakes	3	–	–	–
Corn flakes and grits	4	–	–	–
Mixed breakfast cereals	11	7	0.492 – 2.60	1.55

Table 3. The origin of the samples and aflatoxins contamination

The origin of the sample	No. of samples*	No. of positive samples	Range of aflatoxins contamination (ppb)	Mean of positive samples (ppb)
Bulk	7	–	–	–
Original packaging from Serbia	26	10	0.364–4.08	1.73
Imported products	6	5	0.492–2.36	1.22

*without baby food

The Since content of fumonisins was EU regulated only for corn food samples, and Ridascreen® Fumonisin ELISA test is dedicated for corn and corn-based products, only this type of samples was analyzed for fumonisins (Table 4). The obtained results show much lower fumonisins content than the maximum of 800 ppb permitted by EU regulations.

Table 4. Fumonisins content in corn-based food

Sample Type	Content of fumonisins (ppb)
Corn flakes, bulk	92
Corn flakes, integral, bulk	131
Corn flakes, original packed	< 25
Corn grits, original packed	77
Mixed breakfast cereal, original packed	43

Previous investigations of corn-based food in Serbia [13] showed also the presence of fumonisins in 52.9% samples (out of 17) in a range from 58.2 to 600 ppb. However, it should be pointed out again that the applied tests had a higher detection limit (50 ppb). Thus, if comparison is made at the same detection limit, our result (60% of positive samples) is comparable to the previous finding. The survey for breakfast cereals from the Canadian retail market, indicated 30% of fumonisins positive samples [16].

Judging from the number of aflatoxins positive corn samples (Table 2) there is no correlation between the contents of aflatoxins and fumonisins.

4. Conclusion

The applied ELISA method proved to be simple and fast for the determination of aflatoxins and fumonisins in cereal flakes, grits and breakfast cereals. However, for baby food, there is a need to use a method with lower detection limit. Another shortcoming of this method is that it allows the determination of total aflatoxins and total fumonisins. According to presented results, cereal food in Serbian market is safe, but the percentage of positive samples imposes the need for regular analysis. A growing number of different food cereal products require the development and validation of new analytical methods. The harmonization of Serbian legislation in the field of mycotoxins in food with those in the EU is a necessity.

Acknowledgements

This work was financially supported by SEE-ERA.NET PLUS, Project entitled "Systems to reduce mycotoxins contamination of cereals and medicinal plants in order to preservation native species and traditional products in Romania-Serbia-Croatia area", No. 139 "CROSSMICOTOX".

References

1. Kuiper-Goodman , T . ; *Risk assessment and risk management of mycotoxins in food*. In: Magan. N.; Olsen, M., Eds., *Mycotoxins in food*, Woodhead publishing limited, Cambridge, England, 2004, pp. 3-31.
2. International Agency for Research on Cancer (IARC), Aflatoxins, **2002**, *82* www.inchem.org/documents/iarc/vol82/82-04.html
3. Jakšić, S.M.; Prunić, B.Z.; Milanov, D.S.; Jajić, I.M.; Bjelica, L.J.; Abramović B.F., Fumonisins and co-occuring mycotoxins in north Serbian corn, *Proceedings for Natural Science, Matica Srpska* **2011**, *in press*.

4. International Agency for Research on Cancer (IARC), Fumonisin B1, **2002**, *82* www.inchem.org/documents/iarc/vol82/82-05.html
5. EC (European Commission) Commission Regulation No 1126/2007 of 28 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products, *Official Journal* **2007**, *L 255*, 14–17.
6. EC (European Commission) , Commission Regulation No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins, *Official Journal* **2010**, *L 50*, 8-12.
7. Pravilnik o količinama pesticida, metala i metaloida i drugih otrovnih supstancija, hemioterapeutika, anabolika i drugih supstancija koje se mogu nalaziti u namirnicama Službeni list SRJ, br. 5/92, 11/92, 32/02.
8. Europroxima, Aflatoxin B1 EIA, A competitive enzyme immunoassay for quantitative analysis of Aflatoxins B1 in food and feed samples. www.europroxima.com
9. R-biopharm, Ridascreen Fumonisin, enzyme immunoassay for the quantitative analysis of fumonisin. www.r-biopharm.de
10. Alvito, P.C.; Sizoo, E.A.; Almeida, C.M.M; van Egmond, H.P., Occurrence of aflatoxins and ochratoxin A in baby foods in Portugal, *Food Analytical Methods* **2010**, 3(1), 22-30, doi: 10.1007/s12161-008-9064-x.
11. Villa, P.; Markaki, P., Aflatoxin B_1 and ochratoxin A in breakfast cereals from Athens market: Occurrence and risk assessment, *Food Control* **2009**, *20(5)*, 455-461, doi: 10.1016/j.foodcont.2008.07.012
12. Tam, J.; Mankotia, M.; Mably, M.; Pantazopoulos, P.; Neil, R.J.; Calway, P.; Scott, P. M., Survey of breakfast and infant cereals for aflatoxins B-1, B-2, G(1) and G(2), *Food Additives and Contaminants* **2006**, *23 (7)*, 693-699, doi: 10.1080/12652030600627230.
13. Matić, J.; Mastilović, J.; Čabarkapa, I.; Mandić, A. Mycotoxins as a risk in the grain food, *Proceedings for Natural Science, Matica Srpska* **2009**, 117, 79–86, doi: 10.2298/ZMSPN0917079M.
14. Ghali, R.; Khlifa, K.H.; Ghorbel, H.; Maaroufi, K.; Hedilli, A., Aflatoxin determination in commonly consumed foods in Tunisia, *Journal of the Science of Food and Agriculture* **2010**, *90*, 2347-2351, doi: 10.1002/jsfa.4069.
15. Hernandez-Martinez, R.; Navarro-Blasco, I., Aflatoxin levels and exposure assessment of Spanish infant cereals, *Food Additives and Contaminants Part B-Surveillance* **2010**, *3(4)*, 275-288, doi: 10.1080/19393210.2010.531402.
16. Roscoe, V.; Lombaert, G.A.; Huzel, V.; Neumann, G; Melietio, J.; Kitchen, D.; Kotello, S.; Krakalovich, T.; Trelka, R.; Scott, P.M., Mycotoxins in breakfast cereals from the Canadian retail market: A 3-year survey, *Food Additives and Contaminants Part A-Chemistry Analysis Control Exposure & Risk Assessment* **2008**, *25(3)* 347-355, doi: 10.1080/02652030701551826.