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THE DETERMINATION OF TOTAL AFLATOXINS AND OCHRATOXINA A IN RYE AND BARLEY

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Abstract

The products with a high contamination risk with mycotoxins are: maize, cotton, peanuts, pistachio, and spices. Soya, beans, oats, barley, rice are either resistant or only with moderate susceptibility to contamination with mycotoxins in fields. For these products, the susceptibility grows when they are deposits at high temperature and moisture condition (Hermann, 2002). For consumers protection against de diseases induced by mycotoxins, the qualitative and quantitative control must be done for the products in question, as well as the hygienic measures must be taken against formation of mycotoxins. For mycotoxins determination, we used 2 samples of cereal, one sample of rye and one sample of barley. Mycotoxins tracked in experiments were total aflatoxins and ochratoxin A. Method used for determination of mycotoxins was enzyme-linked immunosorbent assay – ELISA.
***Keywords**: mycotoxins, aflatoxins total, ochratoxin A, ELISA*

Introduction

Mycotoxin is a toxin produced by an organism of the fungus family, which includes mushrooms, molds and yeast. The products with a high contamination risk with mycotoxins are: maize, cotton, peanuts, pistachio, and spices. Soya, beans, oats, barley, rice are either resistant or only with moderate susceptibility to contamination with mycotoxins in fields. For these products, the susceptibility grows when they are deposits at high temperature and moisture condition.

Aflatoxins are a group of secondary metabolites of the fungi species *Aspergillus flavus, parasiticus*. These fungi occur in humid tropical areas and the contamination of vegetable food takes place in

the cultivable countries. Aflatoxins are found as a natural contaminant in several foods, such as peanuts, cotton seed, maize, cassava, rice, cocoa, soy, wheat, sorghum and barley. Aflatoxins belong to the strongest natural occurring cancerogenic substances. Aflatoxin B_1 which is mostly found together with the aflatoxins B_2, G1 and G_2 is the one with the highest toxic importance. Conditions that favor the invasion of corn by A*spergillus flavus* in the field include drought stress or damage to the corn ear by ear worms or other insects, birds, hail, or early frost. High temperatures, high relative humidity around the kernels, and kernel moisture below 30 percent (wet basis) are ideal conditions for fungal invasion of the kernel. The optimum temperature for aflatoxin production in storage is between 25°C and 32°C. (http://en.wikipedia.org/wiki/Aflatoxin).

Ochratoxin A is a mycotoxin produced by various species of *Aspergillus* and *Penicillium*. Ochratoxins contaminate foods (mainly cereals) for humans as well as for cattle. Ochratoxin A production of is connected with temperature, medium humidity and water content of contaminated support. Minimal values of relative humidity for ochratoxin production vary between 0.83 and 0.90, function of mould studied (Hermann, 2002). Optimum temperature for ochratoxin production by Aspergillus ochraceus is 28°C; the synthesis can take place at temperatures between 15 and 37°C. Penicillium veridicantum grows in a large scale of temperature between 4-30°C, at 22% humidity (Weidenborne, 2001).

The moulds producer of ochratoxin A may be able to produce other toxins or may co-operate with other moulds for production different toxins like citrinin or aflatoxin. Can be produced a synergism phenomena with ochratoxin A and can complicate toxic effects analysis, which can not be entirely attributed to ochratoxin A

Ochratoxin A displays hepato-toxic, teratogenic, carcinogenic and immunsuppressives properties. Ochratoxin A was detected in pig blood and kidneys, as well as in human blood and mother's milk (http://en.wikipedia.org/wiki/Ochratoxin_A).

Depending on the toxicity of these mycotoxins in the countries of the EU equal limits are valid for aflatoxins, 2 ppb for aflatoxin B_1 and 4 ppb for all aflatoxins in total (Magan, 2004).

For consumers protection against de diseases induced by mycotoxins, the qualitative and quantitative control must be done for the products in question, as well as the hygienic measures must be taken against formation of mycotoxins.

The aim of the present study was to investigate the incidence and level of contamination with ochratoxin A and aflatoxins total of cereals.

Experimental

Aflatoxins and ochratoxin A can be determined by enzyme-linked immunosorbent assay (ELISA). The basis of the test is the antigen-antibody reaction. The wells in the microtiter strips are coated with capture antibodies directed against anti-mycotoxins antibodies. Standards or the sample solutions, mycotoxin-enzyme conjugate aflatoxin compete for the antibodies are added. Free and enzyme conjugated mycotoxins antibody binding sites (competitive enzyme immunoassay). At the same time, the mycotoxin-antibodies are also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. Enzyme substrate and chromogen are added to the wells and incubated. Bound enzyme conjugate converts the colorless chromogen into a blue to yellow the measurement is made photometrically at 450 nm. The absorbtion is inversely proportional to the aflatoxin concentration in the sample.

One of the advantages of using ELISA-based assays is that the clean-up procedures are not as intensive as for the other analytical techniques (Magan, 2004).

Determination of ochratoxin A from barley and rye: For analysis, samples representatives should be ground and thoroughly mixed in a mixer. 5 grams well-ground feed samples in 12.5 ml of 70% methanol were shaken manually for three minutes. After filtration, 1 ml filtrate was diluted with 1 ml of distilled water. For the quantitative analysis of ochratoxin A, we used the RIDASCREEN ®FAST enzyme immunoassay kit. We used two extracts for each sample.

Determination of total aflatoxin from barley and rye: In the analysis, 2 grams well-ground feed samples mixed in 10 ml of methanol/distilled water (70/30) were shaken manually for three

minutes. Dilute 100µl of the filtrate with 600 µl of the sample dilution buffer. For the quantitative analysis of aflatoxins we used the RIDASCREEN ® Aflatoxin Total enzyme immunoassay kit.

Results and Discussion

For analyses we used rye and barley sample acquired from PLAFAR. Cereals were packed in plastic bags and didn't have visible marks of mould contamination.

Aflatoxins total concentration never exceed 4 ppb, the maximum contamination level fixed by Class number 1050/97/1145/505/2005 (table 1), although the kit allow the aflatoxin total detection at ppt level.

Table 1. Content of total aflatoxins

Ser. No.	Samples	Absorbance			Calculated ppt
		(Mean)	(CV)	(%)	
1	Standard 1	1.124	12.6	100	-
2	Standard 2	1.028	11.6	75.5	49.6
3	Standard 3	1.012	0.2	63.2	149.5
4	Standard 4	0.673	14.3	59.9	450.80
5	Standard 5	0.376	10.2	33.5	1351.00
6	Standard 6	0.210	1.7	18.7	4045.20
7	Rye	1.353	7.0	120.4	**NC**[a]
8	Barley	1.129	18.7	100.4	**NC**[a]

[a] - not calculable

As the result of analysis made we find out that ochratoxin A level from rye sample is high, seeing maxim limit of ochratoxin in raw cereals is 5 ppb, according to Class number 1050/97/1145/505/2005.

In table 2 and in figure 1 are showed detected levels of ochratoxin A in the two samples analyzed.

For rye sample, in the case of 2 parallel extractions equal results were obtained.

Table 2. Content of ochratoxin A

Ser. No.	Samples	Absorbance			Calculated ppb
		(Mean)	(CV)	(%)	
1	Standard 1	0.728	36.7	100	-
2	Standard 2	0.687	15.4	94.4	5.00
3	Standard 3	0.578	2.0	79.4	9.90
4	Standard 4	0.371	5.3	51.0	20.30
5	Standard 5	0.327	26.6	44.9	35.30
6	Rye extract 1	0.615	3.3	84.5	**8.43**
7	Rye extract 2	0.617	29.7	84.8	**8.34**
8	Barley extract 1	0.778	15.5	106.9	**NC**[a]
9	Barley extract 2	0.755	11.1	103.7	**NC**[a]

[a] - not calculable

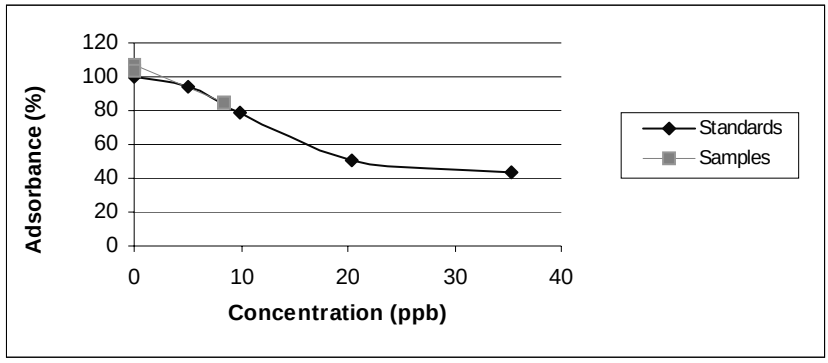

Figure 1. Detected levels of ochratoxin A in analyzed samples

For rye sample, in the case of 2 parallel extractions we obtain equal results.

Conclusions

After analyses, a high level of ochratoxin A was identified in rye samples. Although cereals didn't have visible marks of mould contamination, high quantities of mycotoxins could be found. The regular consumption of cereals contaminated with mycotoxins may

represent a risk factor for the consumer. For consumer protection against diseases induced by mycotoxins, a qualitative and quantitative control of food products is necessary, besides hygienic measures like precaution for preventing mycotoxins formation.

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