Determination of total aflatoxins and aflatoxin B1 content in oleaginous seeds and dried fruits coming from supermarkets and small shops

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Abstract
A very important category of mycotoxins are aflatoxins, made by Aspergillus fungi (A. flavus, A. parasiticus and A. nominus). From all aflatoxins, the following stand out: aflatoxin B1 (AFB1) - most toxic one, aflatoxin B2, G1 and G2 Study was conducted on 25 samples of fruit and seeds originating from supermarkets and small shops around Transylvania. Organoleptic and mycotoxicological tests were performed for all samples. For mycotoxicological testing, RIDASCREEN®FAST Aflatoxin was used, an immunoenzymatic competitive test for quantitative determination of aflatoxins from aliments and cereals and RIDASCREEN AFLATOXIN B1, ELISA test for quantitative determination of Aflatoxin B1 from cereals and fodders. Upon organoleptic testing, 2 samples were noticed to have modified parameters. Mycotoxicological testing revealed 6 samples with higher than normal total aflatoxin content, and 1 sample contained aflatoxin B1 above the upper limit established by the European legislation. The highest total aflatoxin and aflatoxin B1 levels were found in roasted corn (46.5 ppb for total aflatoxins, respectively 4.01ppb for Aflatoxin B1).
Keywords: *fungi, mycotoxins, total aflatoxin, aflatoxin B1, oleagineous seeds*

Introduction

The impact of mycotoxins in food chain is a major global problem, they represent a real danger. Mycotoxins are secondary products of fungal metabolism that can be identified in both food and animal feed. It is known that in small quantities mycotoxins are not dangerous, but public authorities from several countries have set legal limits regarding the presence of mycotoxins in food, in order to prevent the risk of higher contamination. Diseases caused by mycotoxins are called mycotoxicosis and can be acute, chronic or sub chronic. A very important category of mycotoxins are aflatoxins, produced mainly by fungi of the genus *Aspergillus,* the most important species being represented by *A. flavus*, *A. parasiticus* and *A. nominus* and are considered to have a carcinogenic potential in the liver, being also a teratogen and mutagen in both humans and animals. They can enter the body directly through cereals, seeds, spices, fruits and other plant products and indirectly through food products from animals whose contaminated food has led to residues in meat, milk, eggs. and their derivatives (Galvano, 2001). From the category of aflatoxins, the most important are: aflatoxin B1 (AFB1), the most toxic, contributes to the inhibition of messenger RNA synthesis and leads to the formation of liver tumors (hepatocellular carcinoma), aflatoxin B2, G1 and G2 (Masoero, 2009).

Over the past decade, preventing mold contamination in some foods, especially nuts and nuts, has become a real health problem. For prophylactic purposes, the implementation of Good Agricultural Practices (GAP) on regulation in the cultivation, harvesting and storage of fruit and oilseeds is recommended (Yazdanpanah, 2005).

Materials and methods

A number of 25 samples of oleaginous and dehydrated fruits were collected. The samples were collected from hypermarkets, supermarkets and small shops in Cluj and Alba county. Sampling and preparation was done according to the standardized methodology. Oilseeds and fruits were stored either in bulk or in bags. If the samples were collected in bulk, the working method was as it follows: an imaginary division of the lot into an approximately equal number of parts was

made. Randomly, was selected a number of parts, corresponding to the number of partial or elementary samples and was taken at least one sample from each part.

From the mixing and homogenization of the partial (elementary) samples, the general sample was obtained, and from this, using the reduction, by the method of quarters, the average sample was obtained, with a mass of about 100 grams for laboratory analyzes. The samples were packed in new nylon bags, then labeled and sent to the laboratory, where they were recorded and subjected to organoleptic examination.

To perform laboratory tests, was used the RIDASCREEN®FAST Aflatoxin test, a competitive enzyme-linked immunosorbent assay for the quantitative determination of aflatoxins in cereals and food. The test is based on the antigen-antibody reaction. The wells of the plate were labeled with antibodies anti-aflatoxin. Aflatoxin or sample standards, enzyme-aflatoxin conjugate and anti-aflatoxin antibodies were added. Free aflatoxin and enzyme-aflatoxin conjugate compete for antibody binding sites (competitive enzyme-linked immunosorbent assay). At the same time, anti-aflatoxin antibodies are also bound by immobilization of capture antibodies. The unbound conjugate is removed in the washing step. The substrate-chromogen mixture is added to the wells and the bound enzymatic conjugate converts the chromogen into a blue product. Adding the stop solution changes the color from blue to yellow. The measurement is made by spectrophotometry at a wavelength of 450 nm. The absorbance is inversely proportional to the concentration of aflatoxin in the sample.

The samples were processed and analyzed in strict hygienic conditions, respecting the legislation in force so that the results are as conclusive as possible.

Results and discussions

Following the organoleptic examination, changes in appearance, consistency and odor were found in a number of 2 samples: a sample of roasted almonds in which a gray-whitish color was observed on the surface, as well as odor and rancid taste; a mixed fruit sample in which changes in consistency were observed, which were strong and had an aging smell. Following the mycotoxicological examination, it was found that the mixed fruit sample showed an increased level of total aflatoxins, 12.46 ppb, above the maximum limit of 10 ppb allowed by European legislation (EC Reg. No. 1881/2006 and No. 1126/2007).

Out of the total of 25 samples analyzed (Table 1) for the total aflatoxin content, 21 (84%) samples were positive, and out of the total positive samples 24% exceeded the maximum values allowed by European legislation.

Following the analyzes for the content in total aflatoxins, it was found that from the total samples from supermarkets, 9.09% of the samples exceeded the maximum level allowed by European regulations, and in the case of small shops the percentage was 35.71%.

Table 1.

Aflatoxin content in dried fruits and oleaginous seeds (µg/kg)

Specification	Total Aflatoxins - all samples	Total Aflatoxin small shops	Total Aflatoxins supermarket	Aflatoxin B1 - all samples
Nr. of samples	25	14	11	10
% positive samples	84	92.857	72.72	20
Min. and max. of positive samples	1.70 - 46.50	1.70-46.50	1.70- 7.81	2.79 – 4.02

Mean	6.362	8.865	3.176	0.68
Median	4.080	4.430	2.550	0.00
Std. Dev.	9.924	12.664	2.812	1.46
p	≤0.005	≤0.05	≤0.005	≤0.5

Most elevated values of total aflatoxins were identified in the category of roasted peanut-corn-cashew. The maximum value allowed by European legislation for this category is 4 ppb. Out of the total of the 12 samples analyzed in this category, a number of 5 samples exceeded the maximum allowed limits.

Regarding the aflatoxin B1 content, out of the total of the 10 samples examined, only one sample of roasted maize exceeded the maximum level of 2 ppb, allowed by the legislation in force, with a value of 4.01 ppb.

According to a report published by the Iranian Ministry of Health, 7926 pistachio samples were analyzed using the HPLC method from March 2001 to March 2002 in food and drug control laboratories in Iran. The results indicated that 5290 samples (68%) were not contaminated (values <detection limit, LOD) with Aflatoxin B1, 1324 samples (17%) contained Aflatoxin B1 with detection limit 2 µg / kg, 541 samples (5 %) contain AFB1 with values between 2 and 10 µg / kg. According to the report made by the Ministry of Health of Japan, out of 2422 samples analyzed, those between 1972-1989 and sample 2339 (97%) were not contaminated (<LOD), 35 samples (1%) were contaminated with AFB1 with LOD values -10µg / kg, 48 samples (2%) contained AFB1 in an amount greater than 10µg / kg (Yazdanpanah, 2005).

Conclusions

Comparing the samples from supermarkets and small shops, it was found that 9.09% of the total samples from supermarkets respectively 35, 71% of the samples collected from small shops, exceeded the maximum values allowed by European legislation for total aflatoxins;

A contamination with total aflatoxins and aflatoxin B1 was observed both in the non-heat treated and in the processed samples (roasted, seasoned), the thermal factor not decisively influencing the decrease of the aflatoxin content in the analyzed samples.

The study concluded that the level of total aflatoxins was much lower in samples from supermarkets than those from small shops, due to both the origin and storage conditions.

References

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