THE FREQUENCY OF PRESENCE OF AFLATOXIN B1 IN FOODSTUFFS OF VEGETABLE ORIGIN

ABSTRACT: Cereals, nuts and spices are foods that are used in the daily human diet. According to FAO the average consumption of foods of vegetable origin in people’s diet is increasing. Due to inadequate conditions during storage of foods of vegetable origin, there is possibility of contamination by mold that produces mycotoxins. Since the intake of these products in organism has been increased, there is a risk of exposure to mycotoxins and their harmful effect on the consumers’ health. The aim of this study was to determine the presence of aflatoxin B1 in products of vegetable origin (cereals, nuts and spices). Aflatoxin B1 was determined by enzyme-imunochemical method (ELISA), using commercial kit. 38 samples were tested. In 25 analyzed samples, the content of aflatoxin B1 was higher than 1 µg/kg (1 µg/kg is limit of detection). Out of the total number of tested samples, in 18 samples the content of aflatoxin B1 was determined higher than the allowed amount for this product group by the current regulations (2 µg/kg for cereals, 2 µg/kg for nuts and 5 µg/kg for spices).

KEYWORDS: aflatoxin B1, foodstuffs of vegetable origin, ELISA, safety

INTRODUCTION

Cereals, nuts and spices are foods that are used in the daily human diet. According to FAO (Food and Agricultural Organization) the average consumption of foods of vegetable origin in people’s diet is increasing (Food and Agricultural Organization, 2003). Although it is recommended to be used in people’s diet for its nutritional composition, this food can cause adverse effects on human health. Mold can be developed in seed products even before they get on the market due
to inadequate conditions during the storage or inadequate treatment of products. Mycotoxins are secondary metabolic products of mold. The most important molds, which secrete mycotoxins, belong to the genera *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* species (Kabak, 2009). The most important mycotoxins are aflatoxins, ochratoxin A, fumonisin, deoxynivalenol, zearalenone, patulin (Kabak, 2009).

According to FAO (Food and Agricultural Organization, 2003) 30% of the world cereal production is more or less contaminated with mycotoxins. Mycotoxins can occur in all phases of food processing, before the harvest, during the harvest and storage. Food damaged by insects, slow drying and storage of seeds in wet conditions are suitable for mold development (Egmond et al. 2007). Food contamination by mycotoxins can be direct and indirect. In the direct contamination, mycotoxins get into the food as a result of mold growth on food, and in the indirect, contaminated ingredients are used in the food processing or food contains mycotoxin residues (Sinovec et al. 2006). Grain contamination depends on the environmental conditions (moisture content, the relative humidity, temperature, pH value). High soil moisture and grain damage are suitable for mold development (Sinovec et al. 2006).

As a result of consuming food contaminated with mycotoxins, there are serious health problems, which can sometimes be fatal (Boutrif, 1995). Mycotoxins cause a variety of harmful effects on human and animal health, such as hemorrhage, hepatotoxicity, nephrotoxicity, neurotoxicity. In addition, mycotoxins can have teratogenic, mutagenic and carcinogenic effects on the body (Chen et al. 2010). Due to harmful effects on human and animal health, the European Commission (EC) has prescribed the maximum allowed content of several mycotoxins in foods. Maximum allowed content of aflatoxin B1 in foods ranges from 2.0–8.0 µg/kg (EC, 2006).

The best way to prevent mycotoxins development is to prevent mold growth in all phases of production, collecting and transporting, treatment, storage and processing of food. In order to achieve this, it is necessary to control the presence of mycotoxins and mold throughout the food chain.

B1, B2, G1, G2, M1 and M2 aflatoxins represent the highest danger to human health. They are produced by molds of *Aspergillus flavus* and *Aspergillus parasiticus* genus. Under the influence of ultraviolet light, aflatoxins B1 and B2 fluoresce blue and aflatoxins G1 and G2 fluoresce green-yellow (Beltran et al. 2009, Groopman and Kensler, 2005; Malir et al. 2006). Aflatoxin B1 is the most toxic and it is always present in products that contain mycotoxins B2, G1 and G2 (Ilić et al. 2010).

The most commonly used methods for the determination of mycotoxins content in foodstuffs are: reversed phase high pressure-liquid chromatography (RP-HPLC) with UV or fluorescence detector, liquid chromatography (LC) and gas chromatography (GC) with mass spectrometry and enzyme-immunochemical method (ELISA) (Meneely et al. 2011; Sulyok et al. 2010). The most widely used method is the immuno-affinity chromatography with HPLC and the screening method that is used for mycotoxins determination is ELISA method (Krska and Molinelli, 2009).
The aim of this study was to check the frequency of presence of aflatoxin B1 in products of vegetable origin and to check whether content of aflatoxin B1 was present in these products in legally prescribed amounts.

MATERIAL AND METHODS

The aflatoxin B1 content was determined in products of vegetable origin. Samples were purchased on the market of the Republic of Srpska/Bosnia and Herzegovina. They were divided into three groups: cereals (corn, wheat, barley) – 13 samples, nuts (walnut, hazelnut, pistachios) – 19 samples and spices (curcuma, white mustard, pepper) – 6 samples. The samples differed according to their type and the producer. They were prepared according to the instructions of the kit manufacturer. The procedure was the same, only two types of solvents were used. To extract cereals and spices, 70% methanol was used and to extract nuts, 60% methanol was used (Tecna, 2016).

For the determination of aflatoxin B1 content in products of vegetable origin, a commercial kit (Celer AFLA B1, Tecna, Trieste, Italy) was used. The kit contains a set of prepared chemicals. These are standard solutions of following concentrations: 0, 1, 5, 20 and 40 µg/kg, conjugate, a wash solution, solution for color development, a stop solution and 96 wells. The kit is stored at 2–6 °C, according to manufacturer’s instructions (Tecna, 2016).

Aflatoxin B1 content in samples of vegetable origin was determined by ELISA method, measuring the color intensity of the product which appeared in the reaction between the enzyme and added substrate (Šimat, 2010).

Softver Excel spreadsheet for Celer Afla B1 (MA220) was used to measure the content of aflatoxin B1 in the products of vegetable origin.

RESULTS AND DISCUSSION

Table 1 shows the measured absorbance of standard solutions (solutions of well-known concentration) of aflatoxin B1, using an ELISA reader at 450 nm. Aflatoxin B1 standard solutions have the following concentrations: 0, 1, 5, 20 and 40 µg/kg.

Table 2 shows how many samples (cereals, nuts and spices) have the content of aflatoxin B1 ≤1 µg/kg, 1–40 µg/kg and ≥40 µg/kg.

Table 1. The measured absorbance of aflatoxin standard solutions

<table>
<thead>
<tr>
<th>Standard concentration (µg/kg)</th>
<th>0</th>
<th>1</th>
<th>5</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>The absorbance (450 nm)</td>
<td>2.4060</td>
<td>2.0380</td>
<td>0.9890</td>
<td>0.5440</td>
<td>0.4390</td>
</tr>
</tbody>
</table>
Table 2. Aflatoxin B1 content in the analyzed samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number of samples</th>
<th>Aflatoxin B1 content (≤1 µg/kg)</th>
<th>(1-40 µg/kg)</th>
<th>(≥ 40 µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals</td>
<td>13</td>
<td>5 (samples)</td>
<td>4 (samples)</td>
<td>4 (samples)</td>
</tr>
<tr>
<td>Nuts</td>
<td>19</td>
<td>8 (samples)</td>
<td>3 (samples)</td>
<td>8 (samples)</td>
</tr>
<tr>
<td>Spices</td>
<td>6</td>
<td>–</td>
<td>4 (samples)</td>
<td>2 (samples)</td>
</tr>
</tbody>
</table>

According to producer, limit of detection for ELISA Celer Afla B1 (code MA220) is 1 µg/kg for corn, nuts and pistachios and 2 µg/kg for dried fruit and figs (Tecna, 2016).

Out of total 38 tested samples, 13 samples contained less than 1 µg/kg of aflatoxin B1 (limit of detection is 1 µg/kg). Fourteen samples contained more than 40 µg/kg of aflatoxin B1. The content of B1 aflatoxin ranged between 1–40 µg/kg in 11 samples. For samples where content of aflatoxin B1 was ≥ 40 µg/kg, sample extract was diluted (5x) and again determined by enzyme immunochemical method (ELISA).

The highest content of aflatoxin B1 in cereals was 88.29 µg/kg. According to the Regulation of maximum prescribed amounts for certain contaminants in food from Bosnia and Herzegovina, the maximum allowed content of aflatoxin B1 in cereals is 2 µg/kg (Službeni glasnik, 2014). Eight cereals samples contained more aflatoxin B1, than the maximum allowed concentration.

The highest content of aflatoxin B1 in nuts was 94.87 µg/kg. According to the Regulation of maximum allowed amounts for certain contaminants in food from Bosnia and Herzegovina, the maximum allowed content of aflatoxin B1 in nuts is 2 µg/kg. 8 samples contained more aflatoxin B1 than it is allowed by the Regulation (Službeni glasnik, 2014).

The highest content of aflatoxin B1 in spices was 99.64 µg/kg. According to the Regulation of the maximum prescribed content for certain contaminants in food from Bosnia and Herzegovina, the maximum prescribed aflatoxin B1 content in spices is 5 µg/kg (Službeni glasnik, 2014). Two samples contained more aflatoxin B1 than it is allowed by the Regulation.

Czerwiecki et al. (2006) determined mycotoxins content in foodstuffs (cereals and their products, nuts, culinary spices, coffee and dried fruit) available on the Polish market. Mycotoxins content was determined by high pressure liquid chromatography (HPLC) with fluorescence detector. The average content of aflatoxin B1 in the nuts samples was 0.13 µg/kg. The highest content of aflatoxin B1 in the analyzed samples was 7.8 µg/kg (this concentration exceeded the permissible aflatoxin B1 content for nuts, as the European Union recommended, 2 µg/kg. In the analyzed samples of cereals and spices, aflatoxin B1 ranged from 0.02 to 0.4 µg/kg, and an average content was 0.12 µg/kg).

Pluyer et al. (1987) treated peanuts by roasting them in an oven at a temperature of 150 °C, for a period of 30 minutes, and then they monitored what would happen next. Based on the obtained results they concluded that the aflatoxin B1 content decreased for 30–45%. Yazdanpanah et al. (2005) treated pistachios by frying them at a temperature of 90, 120 and 150 °C, for 30, 60 and
120 minutes. It could be noticed that the aflatoxin B1 content decreased for 17–63%, depending on the time and temperature of frying. Ogunsanwo et al. (2004) conducted studies, based on the process of drying seeds at 140 °C for a period of 40 minutes. On that occasion, the content of aflatoxin B1 decreased by 58.8%. Drying at a temperature of 150 °C for a period of 25 minutes, aflatoxin B1 content decreased by 68.5%.

Number of tested samples in this study was small (38), therefore it was expected to get the high percentage (47.37%) of contaminated samples. Considering that the main source of mycotoxins are cereals in the human and animal food chain, it is possible to prevent mold growth and the formation of mycotoxins by applying the measures, good manufacturing practices and the application of HACCP principles. These measures include the selection of varieties resistant to mold, weed control, drying of the grain reducing mechanical damage to a minimum during the harvest, as well as proper drying and storage. In order to protect consumers, it is very important to know the stability of different mycotoxins during thermal processing.

**CONCLUSION**

In the group of tested samples of cereals, 8 samples contained more aflatoxin B1 than it was allowed. In the group of tested samples of nuts, 8 samples contained more aflatoxin B1 than it was allowed. In tested samples of spices, 2 samples contained more aflatoxin B1 than it was allowed. In 25 analyzed samples, the content of aflatoxin B1 was higher than 1 µg/kg (1 µg/kg is limit of detection).

Taking into consideration the small number of tested samples, with a prior suspicion of the presence of mycotoxins, the authors are reserved about the high percentage of contaminated samples in relation to the tested ones.

**REFERENCES**


УЧЕСТАЛОСТ ПОЈАВЕ АФЛАТОКСИНА Б1 У НАМИРНИЦАМА БИЉНОГ ПОРИЈЕКЛА

Весна С. ГОЈКОВИЋ1, Радослав Д. ГРУЈИЋ1, Марко М. ИВАНОВИЋ1, Жељка Р. МАРЈАНОВИЋ-БАЛАБАН2, Драган П. ВУЈАДИНОВИЋ1, Милан С. ВУКИЋ1

1 Универзитет у Источном Сарајеву, Технолошки факултет Зворник, Каракај бб, Зворник 75400, Република Српска, Босна и Херцеговина
2 Универзитет у Бањој Луци, Шумарски факултет Бања Лука, Булевар војводе Степе Степановића 75а, Бања Лука 75000, Република Српска, Босна и Херцеговина

РЕЗИМЕ: Житарице, језграсто воће и зачини представљају намирнице које се користе у свакодневној исхрани људи. Према подацима ФАО-а просјечна по-трошња намирница биљног поријекла повећава се у исхрани становништва. Услед неадекватних услова током складиштења намирника биљног поријекла, постоји могућност да дође до њихове контаминације плијеснима, које производе микотоксине. С обзиром на пораст уноса ових производа у организам, постоји ризик уноса микотоксина и њиховог штетног дјеловања на здравље потрошача. Циљ ovог рада био је да се утврди присуство афлатоксина Б1 у производима биљног поријекла (житарице, језграсто воће и зачини). Одређивање садржаја афлатоксина Б1 вршено је ензимско-имунохемијским методом (ЕЛИСА), коришћењем комерцијалног кита. Испитивано је 38 узорака. Код 25 испитиваних узорака садржај афлатоксина Б1 био је већи од 1 µg/kg (1 µg/kg представља лимит детекције). Од укупно тестираних узорака, у 18 је утврђен садржај афлатоксина Б1 већи од количине дозвољене за ове групе производа у важећим прописима.

КЉУЧНЕ РИЈЕЧИ: афлатоксин B1, намирнице биљног поријекла, ЕЛИСА, безбједност