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THE OCCURRENCE OF AFLATOXINS IN SELECTED SPICES AND DRIED FRUITS

WYSTĘPOWANIE AFLATOKSYN W WYBRANYCH PRZYPRAWACH I OWOCACH SUSZONYCH

Summary. Mycotoxins are toxic substances formed by fungi which are a potential danger for human and animal health. The aim of this study was to determine the contamination with the aflatoxin B₁ and aflatoxins sum B₁, B₂, G₁, G₂ in selected spices and dried fruits available in trade in the Podkarpackie province. The studies confirm the widespread occurrence of aflatoxin B₁ and aflatoxins sum B₁, B₂, G₁, G₂ in spices, spices mixtures and dried fruits, however, in none of the tested samples there was exceeded the permissible concentration of aflatoxin B₁ and aflatoxins sum B₁, B₂, G₁, G₂. The highest content of aflatoxin B₁ was determined in red pepper and universal spices mixture while the smallest in figs and raisins. The monitoring of aflatoxins sum B₁, B₂, G₁, G₂ showed similar results. The highest level was determined in universal spices mixture and in red pepper.

Key words: mycotoxins, aflatoxin B₁, B₁, B₂, G₁, G₂ aflatoxins sum, spices, dried fruits

Introduction

To give food desirable taste and smell, man for centuries has been using plants growing in his environment (BOROWY and KUBIAK 2010). It is well known that herbs and spices are added to many dishes to enhance their organoleptic quality. Of particular importance are dried plant raw materials. In such form, by removing the water to the level hindering the growth of microorganisms and the occurrence of undesirable chemical reactions, they can be stored and used for a longer time. However, dried herbs and spices, despite the presence of antibiotic substances and adverse humidity conditions,

can be colonized by the mould fungi that produce mycotoxins harmful to health (JANDA-ULFIG and ULFIG 2008). The causes of microbiological contamination of dried plants are different, starting from improper conditions of their cultivation, harvesting and drying, and finishing on the wrong packaging of the finished product (JANDA-ULFIG and ULFIG 2008, BOROWY and KUBIAK 2010). It should be noted that the presence of microorganisms in dried herbs and spices is not dangerous to health, although it may cause a significant decrease in the quality of the products. High risk are compounds called mycotoxins, synthesized by the majority of mould fungi (JANDA-ULFIG and ULFIG 2008). Mycotoxins are toxic substances that pose a potential threat to human and animal health (GHALI et AL. 2008, ŁOZOWICKA 2009). The most contaminated spices, both with bacteria and moulds, include black pepper, marjoram, cloves, cinnamon, cumin and red pepper. The most common microorganisms appearing in these spices are bacilli of the species: *Bacillus licheniformis*, *B. subtilis*, *B. pumilus*, *B. breveris*, *B. polymyxa*, and also in smaller quantities *B. coagulans*, *B. alvei*, *B. circulans*, *B. steaerothermophilus*. There are often found pathogenic bacilli of the species *B. cereus*. Among the sporulating bacteria in spices there can appear in the form of spores the filamentous fungi, mostly of the genera *Aspergillus*, *Penicillium*, *Fusarium* or *Rhizopus*, producing mycotoxins of carcinogenic properties for humans and animals (BOROWY and KUBIAK 2010).

The purpose of this study was to determine contamination with aflatoxin B₁ and aflatoxins sum B₁, B₂, G₁, G₂ in selected spices and dried fruits traded in the region of the Podkarpackie province.

Material and methods

The material for tests consisted of food samples taken at points of sale in the Podkarpackie province. The content of aflatoxin B₁ and aflatoxins sum B₁, B₂, G₁, G₂ were determined in accordance with the standard PN-EN 14123:2008 "Foodstuffs. Determination of aflatoxin B₁ and aflatoxins sum B₁, B₂, G₁, G₂ in hazelnuts, peanuts, pistachios, figs, and paprika powder. High performance liquid chromatographic method with post-column derivatisation and immunoaffinity column clean-up" (PN-EN 14123:2008).

An analytical sample was extracted with a solvent solution: methanol-water and a solvent solution: hexane or cyclohexane. The extract of the sample was filtered, diluted with phosphate buffer in phosphate buffered saline (PBS) and fed to the column of the immunoaffinity containing antibodies specific for aflatoxins B₁, B₂, G₁, G₂. The aflatoxins were eluted from the immunoaffinity column with methanol. Quantitative aflatoxin content was determined by high performance liquid chromatography method in the reversed-phase system (RP-HPLC) with obtaining the derivative (PCD) by bromination and fluorimetric detection. PCD was obtained using bromine released from pyridine hydrobromide perbromide (PBPB).

Results and discussion

In spices contaminated by moulds there fairly often occur aflatoxins, even when the signs of mouldiness are not found during sensory tests (BOROWY and KUBIAK 2010). According to the literature (BOROWY and KUBIAK 2010), most frequently the carriers of the very harmful aflatoxin B₁ are: cumin, anise, nutmeg, black pepper and red pepper from India, Indonesia and Malaysia.

The results shown in Table 1 indicate that the content of aflatoxins in the tested spices and dried fruits did not exceed permissible levels (HPL – the highest permissible levels) specified in Commission Regulation (EU) No. 165/2010 of 26 February 2010 (ROZPORZĄDZENIE KOMISJI (UE)... 2010). Current EU regulations allow the concentration of aflatoxin B₁ in spices and spices mixtures at the level of 5.0 µg·kg⁻¹, while in the dried fruits for direct human consumption, at the level of 2.0 µg·kg⁻¹.

Table 1. Content of aflatoxin B₁ in chosen spices and dried fruits (µg·kg⁻¹)
Tabela 1. Zawartość aflatoksyny B₁ w wybranych przyprawach i owocach suszonych (µg·kg⁻¹)

Product Produkt	Number of samples Liczba próbek	Average content Średnia zawartość	The highest content Największa zawartość	The lowest content Najmniejsza zawartość	The highest permissible levels, HPL Najwyższe dopuszczalne poziomy, NDP
Black pepper Pieprz	15	1.61	3.00	0.60	5.0
Red pepper Papryka	8	2.03	4.35	0.30	5.0
Universal spices mixture Mieszanka przypraw uniwersalnych	13	2.23	3.00	1.00	5.0
Ginger Imbir	15	1.57	2.76	1.00	5.0
Turmeric Kurkuma	13	1.67	3.00	1.00	5.0
Raisins Rodzynki	8	0.25	0.60	0.20	2.0
Figs Figi	10	0.20	0.20	0.20	2.0

The analysis of the results in Table 1 indicates that the content of aflatoxin B₁ in all tested samples of spices and dried fruits did not exceed acceptable levels. The average content of denoted mycotoxin was highest in samples of red pepper and universal spices mixture and it was 2.03 µg·kg⁻¹ and 2.23 µg·kg⁻¹, respectively. In other products it was at a lower level, i.e. about 1.6 µg·kg⁻¹. Significantly lower content of aflatoxin B₁ was found in a study conducted by ZINEDINE et AL. (2006), where the average content of this

mycotoxin in black pepper and ginger was $0.09 \mu\text{g}\cdot\text{kg}^{-1}$ and $0.63 \mu\text{g}\cdot\text{kg}^{-1}$, respectively. In addition, these authors found a higher content of the tested mycotoxin in the red pepper, i.e. at the level of $2.88 \mu\text{g}\cdot\text{kg}^{-1}$. For comparison of results there should be also cited the research of AZIZ *et al.* (1998), which showed a high content of aflatoxin B₁ in ginger at the level of $10.0 \mu\text{g}\cdot\text{kg}^{-1}$.

Further analysis of the results contained in Table 1 shows that for samples of dried fruits the average content of aflatoxin B₁ was at a similar level – about $0.2 \mu\text{g}\cdot\text{kg}^{-1}$. However, GHALI *et al.* (2008) showed in their studies the average content of aflatoxin B₁ in dried fruits at the level of $6.4 \mu\text{g}\cdot\text{kg}^{-1}$. Still other results on this issue are quoted by ALGHALIBI and SHATER (2004), who conducted research in Yemen. These authors showed a huge concentration of aflatoxin B₁ in dried raisins ($130\text{-}350 \mu\text{g}\cdot\text{kg}^{-1}$) and figs ($120\text{-}250 \mu\text{g}\cdot\text{kg}^{-1}$). Admittedly, these results strongly differ from the figures in Table 1, where the average content of aflatoxin B₁ in particular fruits did not exceed the value of $0.25 \mu\text{g}\cdot\text{kg}^{-1}$. EU regulations state that the permissible concentration of aflatoxins sum B₁, B₂, G₁, G₂ in spices and spices mixtures should be up to $10.0 \mu\text{g}\cdot\text{kg}^{-1}$, and in dried fruits up to $4.0 \mu\text{g}\cdot\text{kg}^{-1}$.

Detailed analysis of the figures in Table 2 indicates that the highest average content of the studied mycotoxins was found in a universal spices mixture. The average content of the studied aflatoxins in the mentioned samples of spices mixture was significantly

Table 2. Content of B₁, B₂, G₁, G₂ aflatoxins sum in chosen spices and dried fruits ($\mu\text{g}\cdot\text{kg}^{-1}$)
Tabela 2. Zawartość sumy aflatoksyn B₁, B₂, G₁, G₂ w wybranych przyprawach i owocach suszonych ($\mu\text{g}\cdot\text{kg}^{-1}$)

Product Produkt	Number of samples Liczba próbek	Average content Średnia zawartość	Highest content Największa zawartość	Lowest content Najmniejsza zawartość	Highest permissible levels, HPL Najwyższe dopuszczalne poziomy, NDP
Black pepper Pieprz	15	3.03	6.00	1.20	10.0
Red pepper Papryka	9	3.66	7.39	0.60	10.0
Universal spices mixture Mieszanka przypraw uniwersalnych	13	5.31	6.00	3.00	10.0
Ginger Imbir	15	3.23	7.07	2.00	10.0
Turmeric Kurkuma	13	3.33	6.00	2.00	10.0
Raisins Rodzynki	9	0.56	1.20	0.40	4.0
Figs Figi	12	0.55	1.00	0.40	4.0

higher than in samples of other spices and amounted to $5.31 \mu\text{g}\cdot\text{kg}^{-1}$. In other samples the average content was at the level of $3.03\text{-}3.66 \mu\text{g}\cdot\text{kg}^{-1}$. It should be noted that, as in the case of content of aflatoxin B_1 , in none of the tested samples of spices and spices mixtures, was found exceeded permissible concentration of aflatoxins sum B_1 , B_2 , G_1 , G_2 . To compare the results presented one should quote the studies of ZINEDINE et AL. (2006) who showed a much higher average content of the tested aflatoxins in red pepper, i.e. at the level of $5.23 \mu\text{g}\cdot\text{kg}^{-1}$, and lower in the tested samples of black pepper and ginger, at the level of $0.21 \mu\text{g}\cdot\text{kg}^{-1}$ and $1.47 \mu\text{g}\cdot\text{kg}^{-1}$ respectively. In turn, COLAK et AL. (2006) showed the content of the tested aflatoxins in the samples of black pepper at the level of $0.3\text{-}16.7 \mu\text{g}\cdot\text{kg}^{-1}$. Also noteworthy are the studies by CHO et AL. (2008), where the average content of the tested aflatoxins, in the samples of red pepper, was at a low level – $0.87 \mu\text{g}\cdot\text{kg}^{-1}$.

It should be emphasized that in the studies conducted by the authors the average content of aflatoxins sum B_1 , B_2 , G_1 , G_2 in dried fruits did not exceed the permissible concentration approved and was at a similar level, i.e. $0.55 \mu\text{g}\cdot\text{kg}^{-1}$. Comparing these data with the results given in the literature it is clear that they are much lower and more favourable. For example, GHALI et AL. (2008) showed the average content of tested mycotoxins in dried fruits at the level of $9.7 \mu\text{g}\cdot\text{kg}^{-1}$. However, BIRCAN et AL. (2008) showed the presence of tested mycotoxins in dried figs in Turkey within $0.2\text{-}259.46 \mu\text{g}\cdot\text{kg}^{-1}$. In addition, these authors found that an important factor favouring occurrence of aflatoxins was probably the existing drought, high temperature and relatively low humidity of air.

Conclusions

1. The conducted studies confirm the prevalence of both aflatoxin B_1 and aflatoxins sum B_1 , B_2 , G_1 , G_2 in spices, spices mixtures and dried fruits.
2. In none of the samples of the tested spices, spices mixtures and dried fruits the permissible concentration of aflatoxin B_1 and aflatoxins sum B_1 , B_2 , G_1 , G_2 was exceeded.
3. The highest content of aflatoxin B_1 was found in red pepper and universal spices mixture, and the lowest in figs and raisins.
4. Monitoring of the content of aflatoxins sum B_1 , B_2 , G_1 , G_2 was similar. Most of them were found in universal spices mixture and red pepper.

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Streszczenie. Mikotoksyny są substancjami toksycznymi produkowanymi przez pleśnie i stanowią zagrożenie dla zdrowia ludzi i zwierząt. Celem niniejszej pracy było określenie zanieczyszczenia aflatoksyną B₁ oraz sumą aflatoksyn B₁, B₂, G₁, G₂ wybranych przypraw i suszonych owoców znajdujących się w obrocie handlowym na terenie województwa podkarpackiego. Przeprowadzone badania potwierdzają powszechne występowanie zarówno aflatoksyny B₁, jak i sumy aflatoksyn B₁, B₂, G₁, G₂ w przyprawach i mieszankach przypraw oraz w suszonych owocach, jednak w żadnej z badanych prób nie stwierdzono przekroczenia dopuszczalnego stężenia. Największą zawartość aflatoksyny B₁ stwierdzono w papryce i mieszance przypraw uniwersalnych, a najmniejszą w figach i rodzynkach. Podobnie przedstawiał się monitoring zawartości sumy aflatoksyn B₁, B₂, G₁, G₂.

Słowa kluczowe: mikotoksyny, aflatoksyna B₁, suma aflatoksyn B₁, B₂, G₁, G₂, przyprawy, owoce suszone

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