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MYCOTOXINS IN CEREALS AND CEREAL PRODUCTS AS THE RISKS IN FOOD AND THEIR PUBLIC HEALTH SIGNIFICANCE IN THE REPUBLIC OF SRPSKA

MIKOTOKSINI U ŽITARICAMA I NJIHOVIM PROIZVODIMA U REPUBLICI SRPSKOJ KAO RIZICI IZ HRANE I NJIHOV JAVNOZDRAVSTVENI ZNAČAJ

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Ključne reči

mikotoksini u žitaricama,
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zdravstveni rizici

Abstract

Introduction: Controlling mycotoxins in food provides early risk detection since their presence, even in legally permitted concentrations, increases the possibility of health risk occurrences, such as allergy manifestations, and they can have potentially toxic, carcinogenic and genotoxic effects. The aim of the paper is to determine the frequency of the presence of mycotoxins within the group of 'cereals and cereal products' in the Republic of Srpska due to their public health importance and possible health risks, and then to point out the need for monitoring the other mycotoxins specified by regulations.

Material and methods: Examination was conducted on 208 samples of cereals products. Determination of the frequency of the presence of mycotoxins was conducted on a legally prescribed methodology of sampling, chemical analyses and preparation of expert opinions on food safety in accordance with current food regulations. Chi-square test (χ^2) of contingency and descriptive statistical indicators were used for testing the significance of differences in presence of mycotoxins analyzed in samples of cereals and recommended by regulations.

Results: The results indicate that no health defective food samples were determined at tested parameters - aflatoxins B1, B2, G1, G2, total aflatoxins, ochratoxin A i zerealenone. A statistically significant number of samples examined on aflatoxins (6.63%), ochratoxin A(5.65%) and zerealenone (0.25%) was not determined comparing to the other mycotoxins recommended by regulations (deoxynivalenol, 2 and HT-2 toxins, fumonisins) ($\chi^2=0.205$, $p=0.651$).

Discussion with conclusion: A long-term exposure to mycotoxins, even in permitted concentrations, can damage health, meaning that each early detection of mycotoxins as a food risk found through continuous control has had a public health significance for preservation and promotion of population health in the Republic of Srpska.

INTRODUCTION

According to the food regulations, food is considered harmful if it contains mycotoxins exceeding values permitted [1,2]. Mycotoxins as secondary metabolites of molds of the genera *Aspergillus*, *Penicillium* and *Fusarium* are a group of highly toxic, carcinogenic food contaminants of biological origin. The presence of mold in food does not necessari-

ly mean the presence of mycotoxins, and, on the other hand, the absence of visible molds in food does not necessarily mean the absence of mycotoxins because the optimal conditions for growth and development of molds do not have to be identical with mold growth conditions [3,4]. Mycotoxins are the causes of primary and secondary mycotoxicoses. The most common mycotoxin-contaminated foodstuffs are cere-

als, nuts and dried fruits and vegetables, coffee, spices, peanuts, oilseeds, meat products and animal feed [5,6]. Aflatoxins in food are classified in human carcinogens (a Group-1), with a role in the etiology of liver cancer, especially in carriers of HBV antigens [7,8]. Many epidemiological studies have studied carcinogenesis [8]. Aflatoxin B₁ is one of the most powerful mutagens and teratogens with proven carcinogenic effect. Ochratoxin A is a potential factor in the etiology of endemic nephropathy [9]. A serious level of exposure is indicated by ochratoxin A found in 70% of serum samples at respondents in Sweden and about 50% in Germany and Denmark [10]. International Agency for Research on Cancer (IARC) classifies ochratoxin A in a Group-2B possible human carcinogens [7,11]. Zearalenone induces estrogenization and pseudopregnancy at women and prostate cancer at men [12].

Aims of the paper:

1. To determine the frequency of presence of aflatoxins B₁, B₂, G₁, G₂, total aflatoxins, ochratoxin A and zearalenone in samples of cereals and cereal products in the Republic of Srpska from 2010 to 2012;

2. To point out a public health importance of monitoring the presence of mycotoxins analyzed and the other mycotoxins specified by regulations in cereals and cereal products.

MATERIAL AND METHODS

The research was conducted on 208 samples of cereals and cereal products delivered from the foreign and domestic trade in the Republic of Srpska from 2010 to 2012 and divided in 3 subgroups: unprocessed cereals other than maize; cereal products other than corn-based products; corn and corn-based products singled out in a separate subgroup because food regulations provide analysis on fumonisin for this food subgroup only. Aflatoxin B₁ and ochratoxin A were analyzed by photometric method at 450nm using a competitive direct ELISA test (Enzyme-linked immunosorbent assay) in combination with Thin Layer Chromatography with sets/kits ('Europroxim', Netherlands) on the device 'DASSRL', Italy, model A3, TIPO. Total aflatoxins (B₁, B₂, G₁, G₂) and zearalenone were determined by HPLC (High Performance Liquid Chromatography). Determination of food safety was performed in accordance with applicable regulations on food safety [2]. Softwares SPSS 16.0 for Windows; MS Office Word 2007 and MS Office Excel 2007 and MS Office Access 2007 were used for statistical analysis. Chi-square test (χ^2) of contingency was used for testing the significance of differences in presence of mycotoxins analyzed in samples of cereals and products recommended by regulations.

RESULTS

A bar chart 1 shows the percentage of foodstuffs within the group of 'cereals and cereal products' analyzed on mycotoxins. Table 1 shows the data on a number of analyzed samples, and minimum and maximum concentrations of mycotoxins in samples of cereals and cereal products. In samples of unprocessed cereals, determined concentrations of total aflatoxins range from 0.02 to 0.21 $\mu\text{g}/\text{kg}$, and ochratoxin A from 0.20 to 0.95 $\mu\text{g}/\text{kg}$. In samples of the cereals, concen-

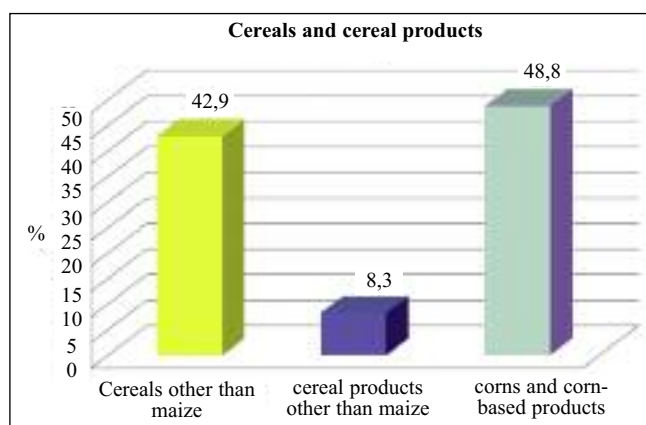
trations of aflatoxin B₁ (<0.05-<0.5 $\mu\text{g}/\text{kg}$), aflatoxin B₂ (<0.05-<0.5 $\mu\text{g}/\text{kg}$), aflatoxin G₁ (<0.05-<0.5 $\mu\text{g}/\text{kg}$), aflatoxin G₂ (<0.05-<0.5 $\mu\text{g}/\text{kg}$), total aflatoxins (<0.5 $\mu\text{g}/\text{kg}$) and ochratoxin A (0.10-1.10 $\mu\text{g}/\text{kg}$). In a sample of wheat flour, determined concentration of zearalenone is 0.50 $\mu\text{g}/\text{kg}$. In samples of corn and corn products, these are determined concentrations of aflatoxin B₁ <0.3 $\mu\text{g}/\text{kg}$, total aflatoxins <0.5 $\mu\text{g}/\text{kg}$ and ochratoxin A 0.49-0.50 $\mu\text{g}/\text{kg}$ (a table 1). Bar chart 2 shows safety in samples of cereals and cereal products in the research period. Table 2 shows the testing of the significance of difference in presence of mycotoxins analyzed in cereals and cereal products and recommended by regulations.

DISCUSSION WITH CONCLUSION

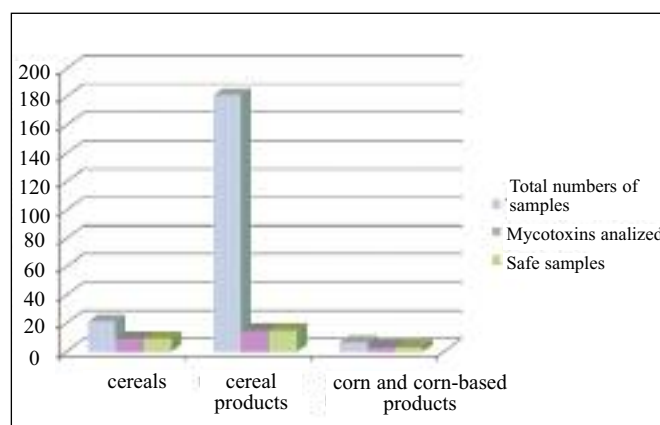
Mycotoxins as contaminants in cereals and cereal products are a public health problem because they affect food safety and can lead to adverse health consequences, especially important to children's health [7,11,13]. This research is justified due to the significance and frequency of cereal consumption as one of the core foodstuffs in all population groups in the Republic of Srpska. Health Survey of the adult population (>18 years of age) of the Republic of Srpska in 2010 shows that white and semi-white bread are consumed by 89,5% of the population, and an average number of slices daily consumed is 4,8. The said research shows that every fifth resident of the Republic of Srpska never thinks about their health when choosing a diet, especially the residents with lower education as well as the ones from rural areas [14]. It is estimated that 500 million poorest people in Sub-Saharan Africa, Latin America and Asia have been daily exposed to aflatoxins and fumonisins by consuming corn-based food and the other cereals [15]. Testing the significance of the difference in presence of mycotoxins analyzed in samples of cereals and cereal products and recommended by regulations shows that a statistically significant number of samples tested for the content of aflatoxins, ochratoxin A and zearalenone was not determined comparing to the number of mycotoxins recommended by regulations in cereals and cereal products ($\chi^2 = 0.205$, $p = 0.651$), as shown in a bar chart 2. In this research, fumonisins were not analyzed in samples of corns and corn-based products, and deoxynivalenol and T2 and HT-2 toxins were not analyzed in the other cereal products (a bar chart 2). Deoxynivalenol and T-2 toxin have cytotoxic and necrotic effects. IARC classifies T-2 toxin in a group-3 human carcinogens [7,15]. The cereals deoxynivalenol-contamination is confirmed by a 6-year research in the Czech Republic [16] and in Poland and Slovakia due to the unsafe samples of the cereals [17]. International Agency for Research on Cancer classified fumonisin B₁ in a Group-2B possible human carcinogens [7,15]. No cereals and cereal product samples posing threat to human health were determined in this research (a bar chart 2). The Food Safety Agency of Bosnia and Herzegovina carried out a risk assessment in 2013 on the presence of mycotoxins in the samples of cereals and products on the basis of laboratory analysis. 19 samples of cereals and processed cereal products were tested for the presence of aflatoxins B₁, B₂, G₁, G₂. Processed cereal-based foods for infants and

young children were tested for the contents of aflatoxin B₁ (30 samples) and ochratoxin A (12 samples). The results of the risk assessment show 16.67% of unsafe samples of the cereals and cereal products due to the increased concentration of aflatoxin B₁. In Bosnia and Herzegovina, a food monitoring and risk assessment for the presence of mycotoxins in 2014 did not comprehend the samples of the cereals and products [18]. Although samples of the cereals and products are safe, the data obtained can serve as a basis for monitoring samples of the cereals and cereal products because a long-term mycotoxin exposure, to even permitted concentrations, damages health, meaning that each early detection of food risk through public health control has had a signifi-

cance for preservation and promotion of population health in the Republic of Srpska. Mycotoxins are of great public health importance due to their thermostability or the ability of not being destroyed by heat treatment, as well as health risks of hepatotoxic, nephrotoxic, neurotoxic and carcinogenic effects. The examination for presence of mycotoxins in the cereals and cereal products points out the general population exposure to mycotoxin contaminants and thus opens the door to further analysis in this field.



Bar chart 1. Percentage of foodstuffs within the group of 'cereals and cereal products' analyzed for the content of mycotoxins from 2010 to 2012



Bar chart 2. Public health control of mycotoxins within the group of 'cereals and cereal products' from 2010 to 2012

Table 1. Determined concentrations of aflatoxins B₁, B₂, G₁, G₂, total aflatoxins, ochratoxin A and zerealenone in samples of cereals and cereal products

Type of food	N of samples	Minimum and maximum concentrations of mycotoxins µg/kg						
		Aflatoxin B ₁	Aflatoxin B ₂	Aflatoxin G ₁	Aflatoxin G ₂	Total aflatoxins	Ochratoxin A	Zerealenone
Cereals *	9					0.02-0.21	0.20-0.95	
Cereals products**	15	0.00-0.21	<0.05-<0.5	<0.05-<0.5	<0.05-<0.5	<0.5	0.10-1.10	0.50
Corn products	3	<0.3				<0.5	0.49-0.50	0.50

* unprocessed cereals (other than maize)

** cereal products (except corn-based products): flour, bread, grits, biscuits and the other milling and bakery products, paste and products, cookies and products, processed cereal-based food and the other cereal products

Table 2. Testing the significance of differences in presence of mycotoxins analyzed in cereals and cereal products and recommended by regulations

Food group	Cereals and cereal products		
REG/Sample	χ^2	df	p
	0.205	1	0.651

Sažetak

Uvod: Kontrola mikotoksina u hrani omogućuje rano prepoznavanje rizika, jer njihovo prisustvo i u zakonski dozvoljenim koncentracijama, povećava mogućnost nastanka zdravstvenih rizika kao što su alergijske manifestacije, a mogu imati potencijalno toksično, kancerogeno i genotoksično djelovanje. Cilj rada je utvrditi učestalost prisustva mikotoksina u grupi hrane „žitarice i proizvodi“ u Republici Srpskoj zbog njihovog javnozdravstvenog značaja i mogućih zdravstvenih rizika, zatim ukazati na potrebu praćenja i drugih mikotoksina predviđenih propisima.

Materijal i metode: Ispitivanje je izvršeno na 208 uzoraka žitarica i proizvoda podijeljenih u 3 podgrupe. Utvrđivanje učestalosti prisustva mikotoksina sprovedeno je na osnovu zakonski propisane metodologije uzorkovanja, hemijskih analiza i izradom stručnog mišljenja o ispravnosti hrane u skladu sa propisima o hrani. Uz pokazatelje deskriptivne statistike, za testiranje značajnosti razlike prisustva analiziranih mikotoksina u uzorcima žitarica i proizvoda i preporučenih propisima korišten je χ^2 test kontigencije.

Rezultati: Rezultati ukazuju da nisu utvrđeni zdravstveno neispravni uzorci hrane za ispitivana obilježja – aflatoksin B1, B2, G1, G2, ukupni aflatoksini, ohratoksin A i zerealenon. Nije utvrđen statistički značajno manji broj uzoraka ispitanih na sadržaj aflatoksina (6.63%), ohratoksina A (5.65%) i zerealenona (0.25%) u odnosu na druge mikotoksine preporučene propisima kao što je deoksinivalenol, 2 i HT-2 toksini i fumonizini ($\chi^2 = 0.205$, $p=0.651$).

Diskusija sa zaključkom: Dugotrajna izloženost mikotoksinima i u dozvoljenim koncentracijama može imati za posljedicu oštećenje zdravlja, pa svako rano prepoznavanje mikotoksina kao rizika iz hrane, kroz kontinuiranu kontrolu, ima javnozdravstveni značaj za očuvanje i unaprijeđenje zdravlja stanovništva u Republici Srpskoj.

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