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Original article

# Occurrence of mycotoxins in talkan: a cereal-based food traditional for Turkic population

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#### **Abstract**

The consumption of cereal contaminated with mycotoxins poses a serious health risk for humans and animals. The present work aims to evaluate the presence of mycotoxins in talkan, a cereal-based food commonly consumed by the Turkic population. The presence of mycotoxins was investigated in a total of 50 samples obtained from Kazakhstan. After a preliminary screening using various ELISA kits, mycotoxins were confirmed and quantified by HPLC-MS/MS method. More than 28% of the samples were positive for at least one mycotoxin. The calculated probably daily intake for adults and children was 20% above the tolerable daily intake for aflatoxin B1 and deoxynivalenol, while it was above 100% for zearalenone, indicating a high risk for the Kazakh population. A total of 12 samples exhibited concentrations above the European maximum level for ochratoxin A, zearalenone and deoxynivalenol, however, these values were within the limits established by the Russia-Kazakhstan-Belarus Customs Union (TR CU 015/2011).

Key words: food-contamination, corn, fungi, HPLC-MS/MS, Kazakhstan

## Introduction

Mycotoxins are toxins produced by filamentous fungi. They are secondary metabolites with no apparent function in the normal metabolism of the fungi. Investigations into the presence of mycotoxins in feed and food began in 1962 with the outbreak of turkey"X" disease where 100000 of turkey poults died in England. The deaths were due to the presence of a toxic substance produced by Aspergillus flavus fungus, the toxin was termed aflatoxin (Nesbitt et al. 1963). Since then, more than 400 different mycotoxins have been reported worldwide with molecular weights ranging between 50 and > 500 Da (Betina 1984). Health effects caused by the consumption of mycotoxins depend on various factors including the type of mycotoxin, its concentra-

tion, the route and duration of exposure, mode of action of the toxin, animal species, gender, age and body weight.

Aflatoxins, which include B1, B2, B3, B4, G1 and G2, are among the main types of mycotoxins, they are primarily produced by the fungi Aspergillus flavus and parasiticus. These mycotoxins affect protein synthesis due to their capacity to bind to DNA (Raisuddin 1993). Aflatoxins have been shown to be extremely potent carcinogens in all animal species investigated and, more recently, the International Agency for Research on Cancer classified them as Group One as they are carcinogenic to humans (IARC 2012). Fumonisins are another group of mycotoxins mainly produced by fungi of the genus Fusarium (Marin et al. 2013). Their concentration in food depends on alkaline solutions, water and temperature, and their concentration in agricultural crops depend on the climatic conditions, latitude, genotype, spoilage and other fungal diseases (Soriano and Dragacci 2004). Known toxic effects of fumonisins on mammalian systems include acting as carcinogenic, hepatotoxic and causative agent in leukoencephalomalacia (Kellerman et al. 1990, Dutton 1996, D'mello et al. 1999) Trichothecenes are a group of sesquiterpenoid mycotoxins that are commonly detected in food and include some of the most potent inhibitors of eukaryotic protein synthesis, interfering in the initiation, elongation and termination steps (D'mello et al. 1999, EMAN 2003). Trichothecene deoxynivalenol is commonly known as vomitoxin, it is mainly produced by Fusarium graminearum and Fusarium culmorum pathogenic fungi that grow on cereals (Marin et al. 2013). Adverse health effects of deoxynivalenol have been reported after acute, short- or long-term administration. It is responsible for a decrease in food consumption (anorexia) and emesis (vomiting). In 1993, the International Agency for Cancer Research (IARC( placed deoxynivalenol in Group 3, not classifiable as to its carcinogenicity to humans (IARC 1993). Zearalenone, previously known as F-2 toxin, is a non-steroidal estrogenic mycotoxin that is biosynthesized through a polyketide pathway by a variety of Fusarium fungi, including F. graminearum (Gibberella zeae), F. culmorum, F. cerealis, F. equiseti, F. crookwellense and F. semitectum, which are common soil fungi found in temperate and warm countries (Bennett and Klich 2003). These fungi contaminate corn and to a lesser extent, barley, oats, wheat, sorghum, millet and rice. Ochratoxin A is a metabolite of different species of fungi including Aspergillus alliaceus, auricomus and Penicillium verrucosum (Pitt 1987, Larsen et al. 2001, Bayman et al. 2002). Ochratoxin A is associated with nephropathy in all animals studied to date. Besides being recognized as nephrotoxic, ochratoxin A also shows hepatoxic, immunosuppressive, teratogenic and carcinogenic behavior (Beardall et al. 1994, Pléstina 1996; Schlatter et al.1996).

Since 1962, mycotoxins have been detected in countries around the world. In particular, aflatoxins have been confirmed in feed and food samples collected in Europe (Marin et al. 2013), Japan (Taguchi et al. 1995), Brazil (Caldas et al. 2002) and Morocco (Zinedine et al. 2006). The occurrence of aflatoxins only in rice has been reported in studies conducted in more than 10 different countries, for example, the United Kingdom, Sweden, Brazil, Canada, Malaysia, India and Nepal (Lim et al. 2015). Between 1993 and 1997, an outbreak of mass mycotoxicosis occurred with fatal consequences in pigs from southern Kazakhstan (Remele 2011). Yet, research on aflatoxins in cereal and cereal-based food products from the Republic of Kazakhstan is scarce. A similar outbreak occurred with fumonisins, which have been reported in cereal and cereal-based food products in Asian countries, such as China (Guan et al. 2011) and Russia (Tutelyan et al. 2013) but no data exist for the presence of fumonisins in cereal, cereal based products or food from the Republic of Kazakhstan. Data for deoxynivalenol in samples collected from Mediterranean countries (Morroco, Italy, Spain and Tunisia) have been reported by Serrano et al. (2012). In 2004, Tutelyan et al. (2013) conducted a research study on this mycotoxin, where a total of 2166 samples of wheat, rye, barley and maize harvested from 1989-2002 from several regions of Russia were analyzed. The Krasnodar region was considered to be the major Fusarium endemic region of Russia as deoxynivalenol was detected in 69% of the samples. A completed review of zearalenone occurrence was published in 2007 (Zinedine et al. 2007), contaminations have been reported in China, which borders with Kazakhstan, where 20% of corn and processed corn samples contained measured levels of zearalenone (Pei et al. 2013). Aflatoxins like fumonisins, deoxynivalenol, zearalenone and ochratoxin A have been detected in cereal and cereal-based food products collected in a large range of countries including Brazil (Caldas et al. 2002), Morocco, Italy, Spain and Tunisia (Serrano et al. 2012).

Regulations that focus on levels of mycotoxins in food and feed have been gradually developed in several countries. In 2003, a total of 100 countries had specific regulations for these toxic compounds (FAO 2004). To date, the maximum limits (MLs) for 13 different mycotoxins or group of mycotoxins in cereal food-based products have been established. These include the most important mycotoxins namely, aflatoxins, fumonisins, deoxynivalenol, trichothecenes, zearalenone and ochratoxin A. In Europe, the MLs are included under Regulation 1126/2007, while those for the Russia-Kazakhstan-Belarus Customs Union are covered by



Table 1. Minimum and maximum permitted levels ( $\mu g/kg$ ) of mycotoxin in cereals or food based-cereal products according to European and Russia-Kazakhstan-Belarus Customs Union legislation.

	Russia-Kazakhstan-Belarus Customs Union	Europe	
Aflatoxin B1	5	0.10	
Fumonisin B1 and B2	4000	200	
Deoxynivalenol	700	200	
Zearalenone	200	20	
Ochratoxin A	5	0.50	

the Technical Regulation of the Russia-Kazakhstan-Belarus Customs Union on the Safety of Grain. Table 1 compiles the MLs established for cereal in Europe and the Russia-Kazakhstan-Belarus Customs Union.

Talkan is a traditional dish commonly consumed by Turko-Mongol people of South Siberia and Central Asia. The dish is based on flour from roasted grains (millet, barley, wheat, oats or corn) that are mashed between two flat-faced stones and is prepared with hot milk or water, salt, butter and sugar. It is particularly consumed for its nutritional properties, being a source of vitamins E and B, and with a high-calorie content and a rational combination of simple and complex carbohydrates that contributed to facile digestiblility (Tarasovskaya and Dzhakova 2012).

Considering the toxicity of mycotoxins, their frequency of detection in cereal and cereal-based food, the lack of information regarding their presence cereal-based food products from the Republic of Kazakhstan and that talkan is a traditional in cereal-based product frequently consumed by Turkic population, this research aims to investigate the presence of mycotoxins in talkan and evaluate the possible health risk from its consumption by Kazakh population with the use of screening (ELISA) and confirmatory (HPLC-MS/MS) methods. This is the first report that discusses the presence of mycotoxins in traditional Turkic food and the possible risk for the population.

# **Materials and Methods**

# Chemicals, reagents and stock solutions

The chemical and chromatographic reagents used were HPLC or analytical grade. Deoxynivalenol, ochratoxin A, zearalenone, aflatoxins B1, B2, G1 and G2 were purchased from Sigma-Aldrich (Steinheim, Germany). Before use, the standard stock solutions (20-200 mg/ml) were prepared in acetonitrile and stored at -20°C in the dark. Deionized water was prepared using a Milli-Q system (Millipore Corporation, USA).

## Sample origins

A total of 50 talkan samples were purchased from local markets across various cities in Kazakhstan (Fig 1).

# Analysis of mycotoxins by ELISA kits

Initially, all samples were analysed using five ELISA kits obtained from EuroProxima (Arnhem, Netherlands) for cereal analysis of aflatoxins (B1, B2, G1 and G2), ochratoxin A, zearalenone, fumonisins and deoxynivalenol. Each analysis was performed in duplicate and following the manufacturer's instructions.

For Aflatoxins detection the kit number 5121AFT was employed. Briefly, 9 mL of 80% methanol was added to 3 g of sample, the mixture was shaken for 30 min, centrifuged for 15 min (1509  $\times$  g) on a centrigude from Ortoalresa Digicen (Madrid, Spain) and 50  $\mu$ L of the supernatant was added to the respective well plate. The absorbance was read at 450 nm using on a Digital and Analog Systems microplate reader from Digital Analog System, model A3 (Rome, Italy). The limit of detection (LOD) achieved with this kit was  $0.5 \, \mu \text{g/kg}$ .

Fumonisins were detected with the kit number 5121FUM, the protocol of extraction was the same as that indicated above for aflatoxins, but the LOD was  $2 \mu g/kg$  for fumonisins.

The kit 5121ZON was employed for zearalenone detection, 20 mL of 84% acetonitrile was added to a 5 g of sample, the mixture was shaken for 30 min, centrifuged for 15 min (1509  $\times$  g) and 50  $\mu$ L of the supernatant was added to the respective well plate, the absorbance of the final extract was read at 450 nm and the LOD with this kit was 12.5  $\mu$ g/kg for zearalenone.

For the presence of deoxynivalenol the kit 5121DON was utilized. To Nineteen ml of Milli-Q water was added 1 g of sample, like for the other Elisa kits, the mixture was shaken for 30 min and centrifuged for 15 min (1509  $\times$  g), then 50  $\mu$ L of the supernatant was added to the respective well plate and absorbance read at 450 nm. The LOD of this kit was 1.5  $\mu$ g/kg.



Ochratoxin A was analysed with the kit 5121OCH. A volume of 10 mL of phosphoric acid and 20 mL of dichloromethane were added to 5 g of a sample. After shaking for 10 min, the mixture was centrifuged for 15 min (1509  $\times$  g) and 12 mL of the lower phase was filtered and concentrated with nitrogen gas. The residue was dissolved in 1.5 mL of buffer and 50  $\mu$ L of the diluted supernatant was added to the respective well plate; absorbance was read at 450 nm and the LOD of this ELISA kit was 1  $\mu$ g/kg for ochratoxin A.

# Analysis of mycotoxins by HPLC-MS/MS

Positive samples obtained with the ELISA kits were sent to the Unit of Molecular Spectroscopy of Central Services (SAI) of the University of A Coruña in Spain, where they were stored at 4°C until analysis. The same procedure was used for the simultaneous analysis of aflatoxin B1, ochratoxin A, zearalenone, fumonisin B1 and B2, and deoxynivalenol in the cereal mixture. The extraction solvent was a methanol-water mixture (80:20). These extracts were stored at -20°C until analysis by HPLC-MS/MS (API 3200, Applied Biosystems, Foster City, CA, USA). The mycotoxins were separated on a Luna C18 HPLC column (150 x 4.6 mm, 5  $\mu$ m) from Phenomenex (Torrance, CA, USA). The mobile phase consisted of solvent A (5 mM ammonium acetate in water) and solvent B (5 mm ammonium acetate in

methanol), which was run in gradient mode at a flow rate of 1 mL/min. The temperature of the column was set at 50°C during the analysis and the injection volume was 20  $\mu$ L. Each mycotoxin was identified based on two multiple reaction monitoring transition (MRM) and the retention time. The LOD declared by the laboratory was 0.9  $\mu$ g/kg for aflatoxin B1, 0.5  $\mu$ g/kg for ochratoxin A, 0.5  $\mu$ g/kg for zearalenone, 0.5  $\mu$ g/kg for fumonisin B1 and B2, and 0.6  $\mu$ g/kg for deoxynivalenol.

#### **Results**

Zearalenone was the mycotoxin most frequently detected; its presence was confirmed in 20% of the talkan samples with the HPLC-MS/MS method; 10 samples were positive and the concentration ranged between 1.3 and 654  $\mu$ g/kg and the mean concentration was 201  $\mu$ g/kg. The analysis with the screening test revealed that only five samples were positive. This was certainly due to the difference on the LODs between the two methods as the LOD of the ELISA kit was 12.5  $\mu$ g/kg and five samples had concentration below this LOD. Regarding health risk, zearalenone was present in the talkan samples between 6 to 33 times above the ML of the EU Legislation (20  $\mu$ g/kg) (Table 3); five samples had zearalenone concentration above the ML of the EU and four samples above the ML estab-



Table 3. TDI (tolerable daily intake) and PDI (probable daily intake) for adult and children calculated from the mean and maximum concentration of mycotoxins measured in talkan samples.

	Mean measured concentration					Maximum measured concentration			
	Adults			Children		Adults		Children	
	TDI	PDI	%TDI	PDI	%TDI	PDI	%TDI	PDI	%TDI
Aflatoxin B1	0.0151	0.004	29	0.006	42	0.004	28	0.006	40
Ochratoxin A	0.122	0.010	8	0.015	12	0.032	27	0.047	39
Zearalenone	$0.25^{3}$	0.287	115	0.419	168	0.934	374	1.363	545
Deoxynivalenol	14	0.179	18	0.260	26	0.596	60	0.869	87

<sup>&</sup>lt;sup>1</sup> Kuiper-Goodman and Scott (1989); <sup>2</sup> EFSA (2010); <sup>3</sup> EFSA (2014); <sup>4</sup> EFSA (2013)

lished in the Technical Regulation of the Russia-Kazakhstan-Belarus Customs Union (200  $\mu$ g/kg) (TR CU 015/2011) indicating a clear food safety problem.

Ochratoxin A and deoxynivalenol were the second and third mycotoxins more frequently detected. Their presence was confirmed with the HPLC-MS/MS method in 9 samples (18% of the samples) and 8 samples, respectively (18% and 16 % of the samples). The concentration ranged between 1.3 and 22.4 µg/kg (ochratoxin A) and between 1.8 and 417  $\mu$ g/kg (deoxynivalenol), and the mean concentration was 7.3 and 125.3  $\mu$ g/ kg for ochratoxin A and deoxynivalenol, respectively. The lowest ML for the investigated mycotoxins is for ochratoxin A with a ML of 0.5 µg/kg in Europe and 5 μg/kg for the Russia-Kazakhstan-Belarus Customs Union. On the other hand, the highest ML is for deoxynivalenol, 3 samples had concentration above the EU legislation (200 μg/kg) and none above the Russia-Kazakhstan-Belarus Customs Union legislation.

The mycotoxin least detected by the confirmatory method was aflatoxin B1; this mycotoxin was measured only in one sample at 2.9  $\mu$ g/kg, the concentration was above the ML set up in Europe (0.1  $\mu$ g/kg) and below the ML set up in the Russia-Kazakhstan-Belarus Customs Union legislation. On the other hand, fumonisins B1 and B2 were not detected in any of the talkan samples, even if the LOD of the HPLC-MS/MS method was as low as 0.5  $\mu$ g/kg.

#### **Discussion**

Zearalenone has been investigated in talkan samples because its presence has been reported in processed food, particularly in that produced from cereals (Lawley et al. 2012). A complete review on the occurrence this mycotoxin in cereals worldwide has been reported by Yazar and Omurtag (2008), which includes data from Italy, Argentina, Morocco, France, Croatia,

Germany, Denmark, Lithuania, Finland, Turkey and China. The concentration range was from not detected to 2564  $\mu$ g/kg. However, the review does not include data for Asian countries located nearby Kazakhstan. Zearalenone production by Fusarium spp. is favored by wet climates, particularly wet and cool weather, and higher occurrence was observed in wheat, rye, rice, corn and sunflower seeds collected from southern humid areas of Russia in 1993, which included Kazakhstan and Uzbekistan (L'vova et al. 1993). This observation could explain the high incidence of zearalenone in talkan samples collected from different areas of Kazakhstan as it was present in 20% of the samples. In another study, it was observed that zearalenone concentration in food was influenced by pH and temperature (100-225°C); the stability of the mycotoxin was investigated using a screw extruder and higher reduction of zearalenone was observed at 120 and 140°C than at 160°C (Ryu et al. 2003). However, results obtained with talkan samples indicated that roasting the grain at 250°C does not guarantee the absence of zearalenone as this mycotoxin was the most frequently detected.

Overall, it could be stated that the range of concentration of zearalenone measured in the talkan samples was similar to those reported in cereal collected and analysed in other countries (Yazar and Omurtag 2008, Pei et al. 2013).

The analysis of ochratoxin A with the ELISA kit gave 12 positive samples; however, the HPLC-MS/MS method confirmed its presence in only nine samples. Something similar occurred with deoxynivalenol which was detected in 12 talkan samples with the screening method and confirmed in 8 samples with the HPLC-MS/MS method. No apparent reason was found to explain the difference between the ELISA and the HPLC-MS/MS results; it is believed that the kits are prepared, validated and tested for cereal flour but not for talkan samples which is a mixture of different roasted cereal and could contain interference that my gave false posi-

able 2. Mean, minimum, maximum concentration ( $\mu$ g/kg) and percentage of positive samples measured in talkan samples by HPLC-MS/MS.

	Mean	Minimum	Maximum	Positive samples (%)	Limit of detection (µg/kg) ELISA	Limit of detection (µg/kg) HPLC-MS/MS
Aflatoxin B1	3.0	3.0	3.0	2	0.5	0.9
Ochratoxin A	7.3	1.4	22.4	18	1	5
Zearalenone	201	1.3	654	20	12.5	0.5
Fumonisins (B1+ B2)	N/A	N/A	N/A	N/A	2	0.5
Deoxynivalenol	125.3	1.9	417	16	1.5	0.6

N/A: Not applicable

tive. These two mycotoxins were detected in 18% and 16% of the samples and for the case of ochratoxin A its concentration was above the MRL permitted by the EU in 9 samples. It was the mycotoxin with the higher number of cases above the UE and Russia-Kazakhstan-Belarus Customs Union MRL. Ochratoxin is recognized as nephrotoxic and has also been shown to have hepatoxic, immunosuppressive, teratogenic and carcinogenic behavior (Beardall et al. 1994, Pléstina 1996; Schlatter et al. 1996). On the other hand, even if deoxynivalenol has EU limit in 200 μg/kg which is 400 times higher than ochratoxin A 3 talkan samples have its concentration above this MRL. Deoxynivalenol is responsible for causing acute temporary nausea, vomiting, diarrhea, abdominal pain and headaches, complete review of its toxicity has been published by Sobrova et al. (2010). Deoxynivalenol was also reported in Russia in 2004, in a survey with 2166 cereal samples harvested from several regions in Russia revealing its presence in 69% of the samples (Tutelyan 2004). In this study the Krasnodar region was considered to be the major Fusarium endemic region of Russia which may explain the high levels of deoxynivalenol and zearalenone in the talkan samples from Kazakhstan, as these two areas seem to have similar weather conditions (Tutelyan 2004). This results also explain the lower incidence of deoxynivalenol in cereal-based products from the Mediterranean area [Morocco, Italy, Spain and Tunisia (n=79)], detected in 5% of the cereal-based products and cereals collected; the concentration ranged between 63.2 and 296  $\mu$ g/kg (Serrano et al. 2012) while the maximum concentration in the talkan samples was 417.

Analysis of the talkan samples with the ELISA kits for aflatoxins (B1, B2, G1 and G2) has reveal of that 10 samples were positive for the presence of one or more aflatoxins, however, the confirmatory HPLC-MS/MS method could only detect aflatoxin B1 in one samples (Table 2). The disparity between the results of the two

methods could be due to the difference in the LODs and the number of aflatoxins which that can be detected; the LOD was  $0.5 \,\mu\text{g/kg}$  for the ELISA and  $0.9 \,\mu\text{g/kg}$  for the HPLC-MS/MS method. Additionally, the HPLC-MS/MS method could only analyze aflatoxin B1, not B2, G1 and G2.

If data obtained for aflatoxin B1 is compared with data reported in other country, as data has not been reported for Kazakhstan, the low incident of aflatoxin B1 in talkan samples (2%, Table 2) is in agreement with data reported on wheat flour samples from China, where only one sample out of 348 contained aflatoxin B1 (Liu et al. 2015). The incident of aflatoxin B1 depends on weather conditions and the type of cereal as these factors affect fungi growth and could explain the high incidence of this aflatoxin in rice samples also collected from China where it was present in 64% of the samples.

Even if aflatoxin B1 was detected in only one sample, its concentration was 29 times higher than MRL acceptable by the EU Regulation but lower than ML established in Russia-Kazakhstan-Belarus Customs Union legislation (Table 1). Aflatoxin B1 is the mycotoxin with the lowest ML established in the EU legislation because it is considered one of the most toxic mycotoxins; it has been shown to be a highly potent hepatocarcinogen in various experiments involving different species such as ducks (median lethal dose (LD50) 0.34-0.56 mg/kg), rainbow trout (LD50 0.81 mg/kg), monkeys (LD 3 mg/kg) and rats (LD 7 mg/kg). In humans, toxicity symptoms include jaundice, ascites, portal hypertension, gastrointestinal bleeding, and possible encephalopathy and fatty degradation of viscera (Corn 1993).

The ELISA analysis indicated 5 samples positive for the presence of fumonisin B1, B2 or B3, whereas the HPLC-MS/MS analysis of these 5 samples gave negative results. Even if the HPLC-MS/MS method was not able to analyze fumonisin B3, it is believed that



the abundance of fumonisin B3 in maize is low as compared to fumonisin B1 and B2 (Scudamore and Patel 2000). Therefore, the absence of fumonisin B1 and B2 guarantee the absence of fumonisin B3. The authors also believe that the absence of positive samples with the HPLC-MS/MS method is due to false positives with the screening method as talkan is a flour mixture of different roasted cereals. On the other hand, even in fumonisin has not been detected in talkan samples its contents in food have been reported worldwide. A review on the global occurrence of this particular mycotoxin in cereal and cereal-based product in the six continents has been conducted by Soriano and Dragacci (2004). Considering the common presence of fumonisin worldwide, the absence of fumonisin in the talkan samples analysed could be due to the fact that the grains are roasted at 250°C before being ground and fumonisin concentration decreases by more than 90% with temperature above 175°C (Bullerman et al. 2002).

# Health risk due to the consumption of contaminated talkan

To evaluate the health risk due to the consumption of contaminated talkan, only data obtained with the confirmatory method have been considered. A total of 14 (28%) samples were positive for the presence of at least one mycotoxin, based on MRL set up by the Russia-Kazakhstan-Belarus Customs Union Legislation (Table 1), the consumption of eight samples (18%) posed a risk for the Kazakh population. However, when the EU MRL was considered the 14 positive samples were non-compliant and potentially toxic. Additionally, it should be highlighted that 6 of these samples contained 3 mycotoxins, 3 samples 2 mycotoxins and 4 samples only one mycotoxin increasing considerable the toxicity effect of the samples. These results suggest that more controls of mycotoxins in talkan samples should be conducted to guarantee the food safety of talkan in Kazakhstan.

On the other hand, the probable daily intake (PDI) was calculated as described previously (Jageret al. 2013) and compared with the tolerable daily intake (TDI) reported for the detected mycotoxin in the talkan samples To calculate the PDI for adults and children, a mean value of 70 kg and 24 Kg of body weight and 0.1 kg and 0.0 5 Kg of talkan consumption was used . Table 3 summarizes the PDI and the percentage of the TDI for adults and children in Kazakhstan (Kuiper-Goodman and Scott 1989, EFSA 2010, EFSA 2013, EFSA 2014). The PDI values for adults and children were above 20% for aflatoxin B1, zearalenone and deoxynivalenol. Considering that mycotoxins accumu-

late in the liver and kidney, these results indicate a high risk for the Kazakh population, particularly when talkan contaminated with zearalenone is consumed. In all instances, PDI of zearalenone represented more than 100% of TDI.

#### **Conclusion**

This research revealed the presence of mycotoxin in talkan, a cereal grain-based food that is prepared using a high temperature and it is a traditional dish for Turkic population. The presence of four mycotoxins was confirmed by HPLC-MS/MS in 28% of the talkan samples and concentrations were above MRL set up by the European legislation. The PDI for adults and children were above 20% for aflatoxin B1 and deoxynivalenol, and above 100% for zearalenone, indicating a high risk for the Kazakh population. It should be highlighted that a small number of scientific articles report the presence of mycotoxins in cereal from Kazakhstan, thus the present study is the first to publish data on the ocurrence of mycotoxins in cereal based-food from Kazakhstan.

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# References

1126/2007/EC COMMISSION REGULATION (EC) No 1126/2007 of 28 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards Fusarium toxins in maize and maize products. Off J Eur Union 255: 14-17.

Bayman P, Baker JL, Doster MA, Michailides TJ, Mahoney NE (2002) Ochratoxin production by the Aspergillus ochraceus group and Aspergillus alliaceus. Appl Environ Microbiol 68: 2326-2329.

Beardall JM, Miller JD, Trenholm HL (1994) Diseases in humans with mycotoxins as possible causes: . In: Mycotoxins in grain: compounds other than aflatoxin. Miler JD, Trenholm HL (eds.) Eagan Press. St Paul. Minnesota, USA, pp 487-539.

Bennett JW, Klich M (2003) Mycotoxins. Clin Microbiol Rev 16: 497-516.

- BetinaV (1984) Mycotoxins. Production, isolation, separation and purification. Elsevier. Amsterdam, The Netherlands.
- Bhat RV, Beedu SR, Ramakrishna Y, Munshi KL (1989) Outbreak of trichorthecene mycotoxicosis associated with consumption of mould-damaged wheat production in Kashmir Valley, India. Lancet 1: 35-37.
- Bullerman LB, Ryu D, Jackson LS (2002) Stability of fumonisins in food processing. Adv. Exp. Med. Biol 504: 195-204.
- Caldas ED, Silva SC, Oliveira JN (2002) Aflatoxins and ochratoxin A in food and risks to human health. Rev Saúde Pública 36: 319-323.Corn M (1993). Handbook of hazardous materials. Academic Press, San Diego, CA, USA.
- D'mello JP, Placinta CM, Macdonald AM (1999) Fusarium mycotoxins: a review of global implications for animal health, welfare and productivity. Anim Feed Sci Tech 80: 183-205.
- Dutton MF (1996) Fumonisins, mycotoxins of increasing importance: their nature and their effects. Pharmacol-Ther 70: 137-161.
- European Food Safety Authority (EFSA) (2010) Scientific Opinion, Statement on recent scientific information on the toxicity of Ochratoxin A, EFSA Panel on Contaminants in the Food Chain. EFSA Journal 8: 1626.
- European Food Safety Authority (EFSA) (2013) SCIENTIFIC REPORT OF EFSA. Deoxynivalenol in food and feed: occurrence and exposure 1. European Food Safety Authority, EFSA Journal 10: 3379
- European Food Safety Authority (EFSA) (2014) Scientific Opinion on the risks for human and animal health related to the presence of modified forms of certain mycotoxins in food and feed 1. EFSA Panel on Contaminants in the Food Chain, EFSA Journal 12: 3916.
- European Mycotoxin Awareness Network (EMAN) (2003)

  European Mycotoxin Awareness Network co-ordinated
  by Leatherhead Food Research Association. UK).
  http://www.lfra.co.uk/eman/index.htm.)
- Guan S, Gong M, Yin YL, Huang RL, Ruan Z, Zhou T, Xie MY (2011) Occurrence of mycotoxins in feeds and feed ingredients in China Journal of Food Agriculture and Environment (JFAE) 9: 163-167.
- Internation Agency for Research on Cancer (IARC) (1993)
  Internation Agency for Research on Cancer. Monographs on the evaluation of the carcinogenic risk of chemicals to humans: some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. 56: 397-444, Lyon, France.
- Internation Agency for Research on Cancer (IARC) (2012). Internation Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risks to humans: chemical agents and related occupations. International Agency for Research on Cancer, 100F: 225-248 Lyon.
- Jager AV, Tedesco MP, Souto PC, Oliveira CA (2013) Assessment of aflatoxin intake in S\u00e3o Paulo, Brazil. Food Control 33: 87-92.
- Kellerman TS, Marasas WF, Thiel PG, Gelderblom WC, Cawood M, Coetzer JA (1990) Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B1. Onderstepoort J Vet Res 57: 269-275.
- Kuiper-Goodman T, Scott PM (1989) Risk assessment of the mycotoxin ochratoxin A. Biomed Environ Sci2: 179-248.

- Larsen TO, Svendsen A, Smedsgaard J (2001) Biochemical characterization of ochratoxin A-producing strains of the genus Penicillium. App Environ Microbiol 67: 3630-3635.
- Lawley R, Curtis L, Davis J (2012) The food safety hazard guidebook. Published by the Royal Society of Chemistry, Cambridge, UK.
- Lim CW, Yoshinari T, Layne J, Chan SH (2015) Multi-mycotoxin screening reveals separate occurrence of aflatoxins and ochratoxin a in Asian rice. J Agr Food Chem 63: 3104-3113.
- LiuY, Lu Y, Wang L, Chang F, Yang L (2015) Survey of 11 mycotoxins in wheat flour in Hebei province, China. Food Addit Contam: Part B 8: 250-254.
- L'vova LS, Orlova N, Bystriakova ZK, Omel'Chenko MD, Remele VV (1992) Propagation of toxigenic fungi and mycotoxins in various grains. Prikladnaia biokhimiia i mikrobiologiia 29: 70-79.
- Marin S, Ramos AJ, Cano-Sancho G, Sanchis V (2013) Mycotoxins: occurrence, toxicology, and exposure assessment. Food Chem Toxicol 60: 218-237.
- Nesbitt BF, O'kelly J, Sargeant K, Sheridan A (1963) Toxic metabolites of Aspergillus flavus. Nature 198: 1056-1058.
- Pei SC, Lee WJ, Zhang GP, Hu XF, Eremin SA, Zhang LJ (2013) Development of anti-zearalenone monoclonal antibody and detection of zearalenone in corn products from China by ELISA. Food Control 31: 65-70.
- Pitt J I (1987) Penicillium viridicatum, Penicillium verrucosum, and production of ochratoxin A. App Environ Microbiol 53: 266-269.
- Plestina R (1996) Nephrotoxicity of ochratoxin A. Food Addit Contam 13: 49-50.
- Raisuddin S, Singh KP, Zaidi SI, Paul BN, Ray PK (1993) Immunosuppressive effects of aflatoxin in growing rats. Mycopathologia 124: 189-194.
- Remele VV (2011) WORKSHOP. Mycotoxin contamination of grain staple crops in Kazakhstan. Kazakh Research Institute for the Processing of Agricultural Products, Astana, Republic of Kazakhstan MycoRed RUSSIA. INTERNATIONAL WORKSHOP. Reduction of Mycotoxins in Production Chains of EU and Russia: Modern Investigations and Practical Features.
- Ryu D, Hanna MA, Bullerman LB (1999) Stability of zearalenone during extrusion of corn grits. J Food Prot 62: 1482-1484.
- Ryu D, Hanna MA, Eskridge KM, Bullerman LB (2003) Heat stability of zearalenone in an aqueous buffered model system. J Agric Food Chem 51: 1746-1748.
- Schlatter CH, Studer-Rohr J, Rasonyi T (1996) Carcinogenicity and kinetic aspects of ochratoxin A. Food Addit Contam 13: 43-44.
- Scudamore KA, Patel S (2000) Survey for aflatoxins, ochratoxin A, zearalenone and fumonisins in maize imported into the United Kingdom. Food Addit Contam 17: 407-416.
- Serrano AB, Font G, Ruiz MJ, Ferrer E (2012) Co-occurrence and risk assessment of mycotoxinsin food and diet from Mediterranean area. Food Chem 135: 423-429.
- Sobrova P, Adam V, Vasatkova A, Beklova M, Zeman L, Kizek R (2010) Deoxynivalenol and its toxicity. Interdiscip Toxicol 3: 94-99.
- Soriano JM, Dragacci S (2004) Occurrence of fumonisins in foods. Food Res Int 37: 985-1000.



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- Taguchi S, Fukushima S, Sumimoto T, Yoshida S, Nishimune T (1995) Aflatoxins in foods collected in Osaka, Japan, from 1988 ot 1992. J AOAC Int, 78: 325-327.
- Tarasovskaya NE, Dzhakova GE (2012) Plants of North Kazakhstan in sports nutrition and recovery malnourished patients. Biological Sciences of Kazakhstan 2: 137-150 (in Rusian).
- The Food and Agriculture Organization (FAO) (2004) Food and Agriculture Organization worldwide regulations for mycotoxins in food and feed in 2003. FAO Food and Nutrition Paper 81. Food and Agriculture Organization of the United Nations, Rome, Italy.
- TR CU 015/2011 (2011) The Technical Regulation of the Russia-Kazakhstan-Belarus Customs Union (CU) on the Safety of Grain (TR CU 015/2011).
- Tutelyan VA (2004) Deoxynivalenol in cereals in Russia. Toxicol Lett 153: 173-179.

- Tutelyan VA, Zakharova LP, Sedova IB, Perederyaev OI, Aristarkhova TV, Eller KI (2013) Fusariotoxins in Russian Federation 2005–2010 grain harvests. Food Addit Contam 6: 139-145.
- Yazar S, Omurtag GZ (2008) Fumonisins, trichothecenes and zearalenone in cereals. Int J Mol Sci, 9: 2062–2090.
- Zinedine A, Brera C, Elakhdari S, Catano C, Debegnach F, Angelini S, Santis De B, Faid M, Benlemlih M, Minardi V, Miraglia M (2006) Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. Food Control 17: 868-874.
- Zinedine A, Soriano JM, Molto JC, Manes J (2007) Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. Food Chem Toxicol 45: 1-18.