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Abstract. Ochratoxin A (OTA), is a mycotoxin, produced by a number *Aspergillus* species and *Penicillium verrucosum*, can be found in several food commodities including cereals, wine, coffee, cacao, spices or dried fruits, milk, meat resulting in a chronic human exposure. According to the toxicological studies, OTA has been shown to be nephrotoxic, hepatotoxic, teratogenic and immunotoxic to animals and human. It is a regulated mycotoxin and maximum limits in a wide range of foodstuffs have been established by Commission Regulation (EC) No 1881/2006. . The aim of our study was to investigate the presence of OTA in cereal derived products and roasted coffee beans and ground roasted beans from Prishtina market. A total of 112 samples were collected from different markets and traditional bazaars during 2016–2017 years, according to the sampling method for the official control of Ochratoxin A laid down in Commission Regulation (EC) No 401/2006. The analytical methods used in this study involved Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS-MS)**.** Our findings show that contamination levels of OTA in all cereal-derived products were lower than the permitted level by European Commission Regulation and Kosovo Food Codex. The frequencies of OTA contamination were 11.62%, 10.05% and 5.95% and the mean concentrations of positive samples were 0.72 μg/kg, 0.84 μg/kg, 0.41 μg/kg for maize flour, wheat-flour, and coffee samples, respectively.

Keywords: Ochratoxin A, maize flour, wheat flour, coffee, LC-MS/MS, Public health Kosovo

Introduction

Ochratoxin A, sometimes named OTA is naturally toxin created by fungal species of the genus Penicillum and Aspergillus (1).
OTA is known as a mycotoxin of stored productts and has strongly linked with Human endemic nepropathy (BEN), and Porcine nepropathy through ingestion of contaminated food and feed with OTA (4,5).

With OTA can be contaminated a large of variety of food products including cereals, cereal by products, wine, coffee, milk, spices, dried fruits, rice, green tea, figs, vegetables, beer etc (7, 8, 9)

The most toxic of the group of ochratoxins is OTA, and is classified as potecial carcinogen at humans (2B group) evaluated by International Agency for research on Cancer (2, 1).

Kosovo Parliament has approved the regulation on contaminats (3) which is in whole text harmonised with European Commission Regulation 1881/2006, where both institutions have set the maximum allowed limit of 3 µg/kg.and 5 µg/kg, for cereal derivatives and for roasted coffee, respectively.

In 10 anniversary of independence, we are still trying to create capacities to monitor the food in the market and have a safer food in our table, to protect public health. The aim of this investigation was to have a mirror of contamination of food marketed in Prishtina. Our study was performed in two years of periods continously 2016-2017.

Sampling

Sampling places in this investigation were chosen randomly from different markets, supermarkets, restaurants, bakeries, flour-mills, traditional bazzars etc. during 2016-2017 years. Cereal by products samples were collected in mostly in flour-mills, but some of the samples were collected also in the bakeries during 2016, in the total 70 samples. For coffee, were collected 42 samples during 2017 also in the Prishtina city. Representative subsamples (10 g) were collected and used in the study. Sampling procedure was done in the harmony with European Commmission regulation 401/2006 (European Commission 2006a). Samples was stored in proper conditions -18^0 C until analysed by LC MS MS at Food and Veterinary Laboratory, in Prishtina.

Sample preparation for Elisa

For Cereals: Grind and mix a representative sample, weigh out 5 g of ground sample and place into a falcon tube 15 ml, add 25 ml of 70% ethanol and shake for 20 min. with a shaker then centrifuge sample for 10 min. At 4.000 rpm, after dilute 1 ml of the obtained supernatant with 1 ml of distilled water, following step is disturbing 50 µl in every microwell in the plate, in double.

For roasted coffee: Grind and mix a representative sample, weigh 5 g of sample and put into a soluble container, added 25 ml of 70% ethanol and shake for 20 min. In a shaker, after this step followed by centrifugation of sample for 10 min at 4.000 rpm, diluting of 50 µl of the obtained supernatant with 950 µl of a 35:65 mixture of ethanol/deionized H2O.

Testing protocol according to manufacturer instructions

First step is to add 50 µl of each Standards in duplicate into different microwells, after add 50 µl of each sample in duplicate into different microwells, followed by adding 100 µl of antibody 1 and mix well by gently rocking the plate manually for 1 min. While incubating period is 30 min at room temperature, following step is to wash 3 times with 250 µl of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels, add 100 µl of Antibody 2 solution, and then the next step is incubating the plate for 30 min. At room temperature, wash the plate 3 time as the same procedure as washing procedure above, add 100 µl of TMB substrate, after incubating 15 min. At room temperature, add 100 µl of Stop Solution to stop the enzyme reaction, the last step is reading the plate at 450 nm wavelength.

Chemicals and standards

Ochratoxin A standard was purchased from Cayman (USA), Immunoaffinity columns MycoSep 229 Ochra were obtained from Romer Lab (Asustria).
Methanol (MeOH), Acetonitrile (ACN), auas ammonia were supplied from LAB SCAN Ltd (Dublin, Ireland).

Determination of OTA

The procedure for OTA extraction from cereals by products is described as follow:
Clean-up: 5 mL from the filtrate were diluted with 40 mL PBS and mixed for 30 sec. Solution was loaded in the MycoSep 229 Ochra column (flow rate 3 mL min−1) and washed once with 10 mL of water. The column allowed to dry by passing air through it. OTA was eluted with 3µL of a solution of methanol. The eluate was then evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved immediately in 500 µl mobile phase and an aliquot was kept at −18°C for the confirmation of the analysis.

Results and Discution

Table 1. The results of OTA contamination in foodcommodities marketed in Prishtina

	n contaminated/n samples				**Mean + SD of the conc. of OTA µg/kg**		
Municipality / Year	Maize flour	Wheat flour	Coffee	Total	Maize flour	Wheat flour	Coffee
Prishtina							
2016	2/17 (11.62%)	3/30(10.0%)	1/30(3.33%)	6/77(7.8%)	0.721±0.112	0.777±0.141	0.321±0.154
2017	0/3(0.0%)	2/20(10.0%)	1/12(8.33%)	3/35(8.6%)	0	0.981±0.112	0.521±0.251
Prishtina Total	2/20(11.62%)	5/50(10.0%)	2/42(5..95%)	9/112(8.04%)	0.721±0.112	0.847±0.112	0.421±0.181

General: A total of 112 foodcommodities samples including 77 from 2016 and 35 from 2017 were analysed for OTA.
Ochratoxin A in maize flour and wheat flour collected from the Prishtina Market were generally in low level.
In total, 8 % (6/77 samples) from the samples collected in 2016 were contaminated, while 9% (3/35 samples) from 2017 were contaminated with ochratoxin A. The mean concentration of OTA in wheat flour samples were 0.78 µg/kg, in maize flour 0.72 µg/kg and in coffee 0.32 µg/kg during 2016, whereas in 2017, in the coffee samples was 0.52 µg/kg, in maize flour samples was 0 and in wheat flour samples was 0.98 µg/kg.
The highest level was in maize flour samples with 1.1 µg/kg.
In this investigation, 9 samples were contaminated with OTA, but none of them exceeded the maximum limit established by Kosovo Food Law and European Commission Regulation on OTA in cereal derivatives and roasted coffee of 3 µg/kg and 5 µg/kg, respectively.
Previous scientific investigation done by (12) has confirmed of the presence of OTA in cereal flour contaminated samples in Prishtina region. But also none of the contaminated samples did not above the maximum limit set by Kosovo Food Law (Official Journal of RKS, 2013) on cantaminants and European Commission Directives 1881/2006.
In the best of our knowledge, there is no investigation of OTA in coffee products marketed in Kosova, so this is the first investigation about of food safety on this foodcommodities.

In Serbia, where more than 30% of our cereal comes from this country, some invstigation were done about OTA in cereal and cereal by products. Last year (10) has made an research investigation with the samples from 2012-2016, from 114 cereal samples, 10.7% maize samples were above the maximum limit set by Serbian Food Codex whereas another investigation were done in 2012 by (11), none of the samples analysed in this research did not exceed the ML.
In conclusion, OTA is not a huge hazard in our country according to our results, but the state agencies which are responsibles to monitor food safety should beginn to analyse for OTA in food commodities, further research is warranted and also to be a national plan of monitoring of mycotoxins including among OTA. Also, needs to be more collaboration between policymakers, bussinesses and consumers to increase of awarenes anf to apply good agricultural practices to reduce the risk from mycotoxins to have a safety food and better and longer life.

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