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SHORT NOTES

Level of ochratoxin A in cereal-flours in the Prishtina region

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Summary. To assess food safety associated with the occurrence of ochratoxin A (OTA) residues in different kinds of flours, a survey was carried out during 2016–2017 in 5 municipalities of the Prishtina region (Prishtina, Gllogovc, Lipjan, Fushë Kosova, and Podujevë). In the present study, a total of 120 flour samples were collected, consisting of domestic and imported samples. The analytical method used in our study to measure the occurrence and concentration range of OTA in the samples was competitive enzyme-linked immunosorbent assay (ELISA) method. A method based on UHPLC-MS/MS was used to confirm the presence of ochratoxin A in samples in which the toxin was detected by ELISA. Out of all the samples examined, nine were contaminated with OTA. The frequency of OTA contamination in 2016 was 5 out of 75 samples (6.7%), and in 2017, 4 out of 45 samples examined (8.9%) were contaminated. OTA was detected in four wheat flour samples (9%), in four maize flour samples (14.8%) and in one rye flour sample (8.3%). The level of OTA ranged from 0.26 to 0.85 μg kg^{-1} in the four positive wheat samples, from 0.77 to 2.75 μg kg^{-1} in the four positive maize samples and the level was 0.77 μg kg^{-1} in the one positive rye sample. None of the contaminated samples exceeded the maximum levels (ML) of 3 μg kg^{-1}, set by to the European Union Regulation and Kosovo Food Codex.

Key words: Ochratoxin A, Cereal Flour, Elisa, UHPLC-MS/MS, Public Health, Kosovo.

Introduction

Ochratoxin A (OTA) is a toxic (poisonous) secondary metabolite produced by moulds, in particular by *Penicillum verrucosum* and *Aspergillus ochraceus*. The two genera that are responsible for producing ochratoxin A (*Penicillum* and *Aspergillus*) in most cases grow in foods and feeds under storage conditions (Bennett and Klich, 2003). Currently, more than 300 mycotoxins have been reported worldwide, of which a small number contaminates food and feed. Ochratoxin A is the most known toxin in the ochratoxin group (Bennett and Klic, 2003; Duarte *et al.*, 2010). *Aspergillus ochraceus* grows at high temperatures, while *Penicillum verrucosum* can produce OTA at low temperatures (Scudamore, 2005). OTA can contaminate different foodcommodities, including wheat, maize, wine, coffee, rye, oats, livestock products such as meat and milk, and human milk (Magnoli *et al.*, 2007; Stoev, 2013; Pinotti *et al.*, 2016). OTA is resistant to processing under high temperatures, so OTA can be found in roasted coffee and flours and is difficult to remove under normal cooking conditions (Hussein and Brasel, 2001). Cereals and related by-products are commodities that, under specific conditions (insufficient drying, condensation, heat, moisture, insects etc.) can support fungal growths that are able to produce OTA in the field, after harvest or in storage (Eskola *et al.*, 2001; Ramesh and Siruguri, 2003; O`Brien and Dietrich, 2004; Paterson and Lima, 2010; Dehelean *et al.*, 2011).

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Commodities such as bread and other food items with cereals and cereal derivatives in their ingredients are commonly consumed in large amounts in Kosovo, and the presence of OTA in these commodities may result in considerably increased human exposure to this mycotoxin. In 2016, in Kosovo 134,886 ha were planted with cereals, of which 97% were planted with wheat and maize, two crops that have long occupied the majority of cultivated agricultural land and only 396 ha were planted with rye. The self-sufficiency rate for wheat in Kosovo is 63.3% and 69.9% for maize, and the remaining supply comes from import (Green report MAFRD, 2016). Human exposure to ochratoxin A has been indicated in several European Countries, shown by its presence in blood, urine and milk (EC, 2015; Heyndrickx *et al.*, 2015). Ochratoxin A causes renal toxicity, nephropathy, renal cancer and immunosuppression in several animal species, and independent clinical studies and some studies have indicated ochratoxin A as the possible the main cause of the human disease Balkan endemic nephropathy which appears mainly in the Balkan peninsula (O`Brien and Dietrich, 2004; Grollman and Jelakovic, 2007; Pfohl-Leszkowicz A, 2009).

The European safety authority (EFSA, 2010), European Commission, U.S. Department of Health and Human Services (National Toxicology Program), Joint FAO/WHO Expert Committee on Food Additives (JECFA), and the International Agency for Research on Cancer (IARC) have increased the rate of data availability from all over the world. According to evaluation of the available data, it has been proposed to set maximum limits for OTA in food for cereals and cereal derivatives and other foodcommodities (IARC, 1993; JECFA, 1991, 1995, 2002, 2007; 41 EC, 2002; Walker, 2002; EFSA, 2006). The Food and Veterinary Agency of Kosovo (REGULATION (GRK) No. 43/2013) adopted the European Union limit of 3 μg kg^{-1} for ochratoxin A in cereal derivatives. Because there are limited data available in our country, this review aims to critically evaluate the occurrence of OTA in different commodities intended for human consumption. The goal of this study was to assess the level of OTA in different kinds of flours, including domestic and imported samples in retail markets in the Prishtina region (including 5 municipalities).

Methods

Samples

A total of 120 cereal flour samples were collected from flour mills, supermarkets, processing factories, sales points, border check points etc. over a 2-year period between May–November 2016 and February–September 2017 from five major municipalities of the Prishtina region (Prishtina, Gllogovc, Lipjan, Fushe Kosova, and Podujeve). In 2016, a total of 75 different locally and imported flour samples were collected: 50 wheat flour, 20 maize flour and 5 rye flour, in 2017 the collected research materials consisted of 25 wheat flour, 15 maize flour and 5 rye flour samples. The 500 g samples of wheat flour, maize flour and rye flour were bought in 5 municipalities of the Prishtina region and were placed into a sterile sachet and brought immediately to the Food and Veterinary Laboratory for analysis, where they were stored until use. Sampling procedures were compatible with European Directive (EC) No 401/2006. Each sample was kept in cold conditions until analysis (Krska *et al.*, 2008; Yazdanpanah, 2011).

ELISA screening method

OTA screening tests were performed at the Kosovo Food and Veterinary Agency in Prishtina, Kosovo. The assessments of OTA amounts were conducted using the Bioo Scientific Ochratoxin A (Catalogue #: 1036, USA) ELISA test kits. OTA-positive samples detected using the ELISA test were further quantitatively analyzed by UHPLC-MS/MS in a reference laboratory (EURL) for mycotoxins in the Veterinary and Agrochemical Research Center VAR-CODA-CERVA, Brussels, Belgium.

Sample preparation

ELISA analysis were carried out according to manufacturer instructions, 5 g of each sample was weighed and placed in a container, followed by the addition of 25 mL of 70% ethanol and shaking over 20 min with a shaker at room temperature. We then we put the samples into a centrifuge for 10 min at 2000 g. Then, 1 mL of the obtained supernatant was diluted with 1 mL of distilled water, and 50 μL of the diluted supernatant was dispersed per well for the test.

Elisa testing protocol

Microtiter strips sufficient in amount for the standard and specimens were placed on the plate, and 50 μL each of ochratoxin A standards were added in duplicate into different wells (standards were added into the plate in the order from low concentration to high concentration). A 50 μL amount of each of the

samples was also dispersed in duplicate into different wells. After that, into each well was added 100 μL of antibody #1 and solutions were mixed well by gently rocking the plate manually for 1 minute. The plate was incubated for 30 min at room temperature (20–25^{0}C). Then, the plate was washed 3 times with 250 μL of 1X Wash Solution. After the last wash, the plate was inverted and gently dried on paper towels. Following this step, the solution containing 100 μL of antibody #2 was dispersed into each well and incubated at room temperature for 30 min avoiding direct sunlight and cold bench tops during the incubation. A cover was used for the microtiter plate in this step. After this period of incubation, the plate was washed 3 times with washing solution in the same way as in first wash. Then, 100 μL of TMB (tetramethylbenzidine) substrate was added in the following step and the following incubation period was for 15 min at room temperature. Finally, 100 μL of Stop Buffer was disbursed to stop the enzyme reaction, and the absorbance was assessed at a 450 nm wavelength (Tecan reader, Infinite 200Pro, Nanoquant, Austria). The results obtained via reading over a calibration curve were multiplied by the dilution factor, which was 10. The colour development was inversely proportional to the OTA concentration in the sample. The concentration of OTA was calculated from the calibration curve which was obtained using standards with the following concentrations: 0, 15, 30, 60, 120 and 240 ng/L. LOD (Limit of detection) was 0.15 μg/kg. The analytical quality of the ELISA method was assured by the use of certified reference material (CRM) *Sigma Aldrich nr. 0476983-7*.

The validation parameters (Table 1) were calculated and expressed using the European Official Decision procedure for screening methods and their values were in accordance with recommendations given in the Commission Decision (European Commission, 2006).

Confirmatory methods

Reagents and materials

The reference method by Kiebooms J.A.L. 2016were used to confirm the OTA in cereal flours. ULC-MS grade glacial acetic acid (AcOH), ULC-MS grade ammonium acetate (AmAc), ULC-MS grade methanol (MeOH), ULC-MS grade acetonitrile (ACN), AR-grade acetone (DMK), AR-grade monoethylene glycol (MEG), HPLC-S grade ACN, AR-grade AcOH and AR-grade isopropanol (IPA) were purchased from Biosolve (Valkenswaard, The Netherlands). Purified water was obtained from a Milli-Q system (Millipore, Overijse, Belgium). All fractional percentages of solvent concentration are volumetric percentages unless stated otherwise. Additionally, 2 mL disposable syringes, 2-mL disposable polypropylene (PP) centrifugation tubes, 50 mL PP extraction tubes with screw taps, anhydrous AR-grade magnesiumsulfate ($MgSO_4$) and AR-grade sodium chloride (NaCl) were purchased from VWR (VWR, Haasrode, Belgium). Then, 2 mL amber glass vials with and without inserts were purchased from Waters (Waters Corp., Milford, MA. USA). Additionally, 15 mm syringe filters with a 0.2μm regenerated cellulose (RC) membrane were purchased from Phenomenex (Phenomenex, Utrecht, Netherlands).

OTA was purchased in powder form from Romer Labs (Romer Labs, Tulln, Austria). Thispowder were dissolved in pure ACN (ULC-MS-grade). From this mixture, a working solution was used to prepare the

Table 1. Validation of the method was performed with spiked CRM.

OTA spiked (ppb) (n = 5)	OTA found (μg kg^{-1})	Recovery (%)	Standard deviation SD	Variation coefficient (%)	LOD Limit of detection	LOQ-Limit of quantification
1	1	100	0.0	0.0	1	1
2	2.1	105	0.06	2.85	6.3	21
3	3.2	106.6	0.15	4.68	9.6	32
4	3.90	97.5	0.2	5.12	11.7	39
6	5.85	97.5	0.3	13.675	17.55	58.5

Table 2. Validation of the method of LC MS MS.

	Spikelevel (µg kg^{-1})	Rec (%)	RSD_{RW} (%)	LOQ (µg kg^{-1})	Extended measurement uncertainty (k=2)
OTA	2.5	85	10	1	35
	5	85	10		
	7.5	85	11		

Table 3. Results by year of sample collection.

Year	Number of samples	Number positive	% positive	95% c.i.*	Min (µg kg^{-1})	Max (µg kg^{-1})
2016	75	5	6.7	2.9–14.7	0.26	2.75
2017	45	4	8.9	3.5–20.7	0.43	0.97
Overall	120	9	7.5	4.0–13.6	0.26	2.75

* 2x2 comparison using Fisher Exact test (2 tail): P-value = 0.9084 (not significant).

calibration curve and quality controls and to spike the recovery quality control, which was prepared in a solution of AcOH acidified aqueous methanol (80 vol% MeOH, 19 vol% water, 1% AcOH) and stored at -20°C in the dark. LOD was 0.3 µg kg^{-1}.The concentrations set for OTA in these solutions was 200 ng mL^{-1}. Their concentration was verified by an in-house validated UHPLC-MS/MS (ultra-high performance liquid chromatography-tandem mass spectrometry) method using certified reference solutions.

Sample preparation

For the extraction, 5 g (± 0.02 g) sample was placed into a disposable 50 mL PP centrifuge tube with a screw cap. Then, 5 mL of an aqueous extraction solvent (100 g of NaCl dissolved in 990 mL of water and acidified with 10 mL of AR-grade AcOH) and 20 mL of an organic extraction solvent (490 mL ACN added to 500 mL of IPA and acidified with 10 mL% of AR-grade AcOH) was added, and the tube was shaken on a Reax 2 overhead shaker (VWR, Haasrode, Belgium) for approximately 60 minutes. A mixture of 6g $MgSO_4$/1.5 g NaCl was added after which the tube was vigorously shaken by hand for 1 minute. After centrifugation for 5 minutes at 16800g 2 mL of the upper organic layer was filtered through a 0.2 µm RC filter.

LC-MS Conditions

The UHPLC system consisted of an Acquity UPLC® H-Class system (Waters, Milford, MA, USA) equipped with a quaternary solvent manager and a flow through needle sample manager. A XEVO® TQ-S (Waters, Manchester, UK), equipped with an electrospray (ESI) source, was used as the detector. Experiments were carried out in the multiple reaction monitoring modes (MRM). The cone voltage and collision energy were optimized for each component during tuning by the infusion of standard solutions at a flow rate of 20 µL min^{-1} into a column with a flow rate of 200 µL min^{-1} of 50% methanol/water buffered with AmAc (5 mmol) and AcOH (0.05%) using the integrated syringe pump. At least two product ion transitions for each analyte were selected in the final method. The most abundant product ion was used for quantification, unless it was produced due to non-specific fragmentation (e.g. Water loss), while the secondary product ion was used for confirmation. The infusion experiments were also used to determine whether the positive or negative mode gave the highest signal to noise ratio. The source temperature was set at 150°C, while the capillary solvation heater was set at 450°C. The capillary voltage used was 0.5 kV, both in positive and negative modes. The drying gas was nitrogen at a flow rate of 1000 L h^{-1}. Both quadru-

poles were operated at unit mass resolution, and the collision cell was operated at an argon pressure of 3.5 $\times 10^{-3}$ mbar. These parameters were used for analyses in both the positive and negative ion mode. The LC-MS was operated using MassLynx® 4.1 software.

For the LC-MS procedure, 0.2 µL of the extract obtained was injected on a Phenomenex® Kinetex-pentafluoro- phenyl phase(PFP) 2.1 × 100 mm, 1.7µm column and analyzed using a linear gradient starting at 2.5% solvent B (ULC-grade methanol buffered with 5 mmolAmAcand 0.05% AcOH) and 97.5% solvent A (water buffered with 5 mmol AmAc and 0.05% AcOH) and rising to 99% B in 15 minutes. The column was then rinsed with 99%B for 1 minute and equilibrated for 4 minutes with 2.5% solvent B.

The response was calculated using the chromatographic peak area. The two main criteria for positive identification were (a) retention time (must lie within +/- 2.5%) and (b) ion ratios defined as the qualifier area /the quantifier area. Acceptable results had to fall within +/- 20% if the ratio was between 0.5 and 1or within +/- 25% if the ratio was lower than 0.5. The standards were used to establish reference criteria during a sample run.

Results

Results by screening method

The occurrence and levels of OTA in flour samples are presented in Tables 4 and 5. Altogether, the toxin was detected in 9 flour samples, corresponding to 7.5% of the total samples examined. Considering the European Commission and Kosovo Food Codex limits, none of the contaminated flour samples had OTA concentrations in excess of the maximum tolerance limit of 3 µg kg^{-1}. As shown in Table 4, contaminated samples contained OTA levels between 0.26 and 2.75 µg kg^{-1}. Levels in the samples collected from May to November 2016 ranged between 0.260 and 2.75 µg kg^{-1}, while levels in the samples obtained from February to September 2017 ranged between 0.43 and 0.97 µg kg^{-1}. In our study, the minimum levels of OTA in the flour samples was 0.26 µg kg^{-1}, while the maximum level was 2.75 µg kg^{-1}. The data also shows that OTA levels in the flour were affected by the geographical region as is presented in Table 5, by the variation in the proportion of OTA contaminated samples collected in different municipalities of the Prishtina region. In the 2016 sample set, the higher proportions of samples contaminated with OTA in flour were found in Lipjan municipality 15.0% (3/20), and Prishtina 10.0% (2/20), while there were no OTA contamination of sample from Fushe Kosove, Podujeve and Drenas. During 2017, Prishtina was the municipality with the most positive cases (3) while the Podujeve, Lipjan and Fushe Kosova municipalities had no contaminated samples, and Drenas had 1 positive case. The levels of OTA in the contaminated samples collected in 2016 and in 2017 ranged from 0.26 to 0.85 µg kg^{-1} in wheat flour and from 0.67 to 2.75 µg kg^{-1} in maize flour, and the level was 0.77 µg kg^{-1} in the single contaminated rye flour sample, as shown in Table 3. In terms of the regional distribution of positive samples during 2016 and 2017, Prishtina was the municipality with most of the positive samples 5 or (55.6%) and the highest proportion of contaminated samples (5/35 or 14.3%). Samples that are positive for OTA in Elisa analysis

Table 4. The amounts of ochratoxin A in the different flour sample.

Type	Number of samples	Number positive	% positive	95% confidence interval*	Min (µg kg^{-1})	Max (µg kg^{-1})
Maize flour	27	4	14.8	5.9 - 32.5	0.67	2.75
Rye flour	12	1	8.3	1.5 - 35.4	0.77	0.77
Wheat flour	81	4	4.9	1.9 - 12.0	0.26	0.85
Grand Total	120	9	7.5	4.0 - 13.6	0.26	2.75

* 95% confidence intervals for prevalence estimates are calculated using the Wilson score intervals method (Wilson, 1927; Wallis, 2013) as provided in the free online statistical toolbox at OpenEpi.com. This method provides exact, non-symmetrical confidence intervals that are robust even when sample size is small and/or the prevalence is close to 0 or 100%.

Table 5. The OTA contamination of flour in each municipality in 2016 and 2017.

Municipality / Year	No. contaminated/No. samples				Mean + SD of the conc. of OTA μg kg^{-1}		
	Maize	Rye	Wheat	Total	Maize	Rye	Wheat
Gllogovc							
2016	0/2 (0.0%)	0/1 (0.0%)	0/7 (0.0%)	0/10 (0.0%)	0	0	0
2017	1/1 (100.0%)	0/1 (0.0%)	0/6 (0.0%)	1/8 (12.5%)	0.97 ± 0.16	0	0
Gllogovc Total	1/3 (33.3% I)	0/2 (0.0%)	0/13 (0.0%)	1/18 (5.6%)	0.967 ± 0.16	0	0
Fushe Kosova							
2016	0/3 (0.0%)	0/1 (0.0%)	0/6 (0.0%)	0/10 (0.0%)	0	0	0
2017	0/1 (0.0%)	0/1 (0.0%)	0/5 (0.0%)	0/7 (0.0%)	0	0	0
Fushe Kosova Total	0/4 (0.0%)	0/2 (0.0%)	0/11 (0.0%)	0/17 (0.0%)	0	0	0
Lipjan							
2016	1/4 (25.0%)	1/2 (50.0%)	1/14 (7.1%)	3/20 (15.0%)	2.75 ± 0.28	0.77 ± 0.05	0.85
2017	0/2 (0.0%)	0/1 (0.0%)	0/5 (0.0%)	0/8 (0.0%)	0	0	0
Lipjan Total	1/6 (16.7%)	1/3 (33.3%)	1/19 (5.3%)	3/28 (10.7%)	2.75 ± 0.28	0.77 ± 0.05	0.85
Podujeva							
2016	0/4 (0.0%)	0/1 (0.0%)	0/10 (0.0%)	0/15 (0.0%)	0	0	0
2017	0/1 (0.0%)	0/1 (0.0%)	0/5 (0.0%)	0/7 (0.0%)	0	0	0
Podujeva Total	0/5(0.0%)	0/2 (0.0%)	0/15 (0.0%)	0/22 (0.0%)	0	0	0
Prishtina							
2016	1/5 (20.0%)	0/2 (0.0%)	1/13 (7.7%)	2/20 (10.0%)	0.77 ± 0.20	0	0.26 ± 0.16
2017	1/4 (25.0%)	0/1 (0.0%)	2/10 (20.0%)	3/15 (20.0%)	0.67 ± 0.06	0	0.45 ± 0.030
Prishtina Total	2/9 (22.2%)	0/3 (0.0%)	3/23 (13.0%)	5/35 (14.3%)			
Overall	4/27 (14.8%)	1/12 (8.3%)	4/81 (4.9%)	9/120 (7.5%)			

should be confirmed by chromatographic methods (Shephard, 2016). None of the contaminated samples exceeded the maximum residue levels (MRL) of 3 μg/kg set according to the European Union Regulation and the Kosovo Food Codex.

Confirmation of qualitative results by reference laboratory

Eight positive samples were analyzed by UHPLC-MS/MS in the European Union Referent Laboratory (EURL) for mycotoxins in the Veterinary and Agrochemical Research Center VAR-CODA-CERVA, Brussels, Belgium (Kiebooms *et al*., 2016). OTA toxin was detected by this lab in only one sample. Only one sample of maize flour had a contamination of 2.8 μg kg^{-1} with uncertainity measurement of 1.1 μg kg^{-1} which is close to the 3 μg kg^{-1} MRL. This finding is compliant with the results gained by ELISA analysis, as the others were not quantified because they were below of the limit of quantification.

Confirmatory results for suspect samples analysed by UHPLC-MS/MS are shown in Table 6.

Discussion

This is the first survey in Kosovo to determine the level of OTA contamination in flour samples derived

Table 6. Confirmatory results.

Referentie CODA-CERVA	Reference client	Concentration of OTA (μg kg^{-1})*	Measurement uncertainty (μg kg^{-1})
E-2017_447	S1	2.8	1.1
E-2017_448	S2	< RL	/
E-2017_449	S3	< RL	/
E-2017_450	S4	< RL	/
E-2017_451	S5	< RL	/
E-2017_452	S6	< RL	/
E-2017_453	S7	< RL	/
E-2017_454	S8	< RL	/

* RL (reporting limit) = 1 μg kg^{-1}.

Figure 1. Sampling localization (loc.): 1) Prishtina, 2) Podujeve, 3) Gllogovc, 4) Lipjan, and 5) Fushe Kosova.

from wheat, maize and rye. Milling had been reported to substantially reduce the concentration of OTA in wheat flour (Cheli *et al.*, 2017), hence the higher incidence of OTA in whole wheat flour was reasonable. In Serbia, such research performed on wheat flour samples showed that OTA contamination in all samples analyzed were compatible with EU and Serbian regulations for maximum residue levels (Škrbić *et al.*, 2012). Another, study on OTA in Serbia, published in 2017 showed that out of 114 samples collected during 2012–2016, 29% contained OTA, and 10.7% of the maize flour exceeded the MRL. Rye samples had the highest record level of 8.80 μg kg^{-1} (Torović, 2017). In Croatia, OTA was detected in 75.8% or (74/92) of wheat samples and 33.3% (17/51) of corn samples, at detected levels from 0.02–160.0 μg kg^{-1} in wheat samples and 0.02–40.0 μg kg^{-1} in maize samples. According to the results of a study in Croatia, all 33 wheat samples from Slavonski Brod and 9 out of 11, (81.8%) of corn samples were positive for OTA, and the highest mean values of ochratoxin A detected were 38.8 and 27.2 for wheat samples and 20.0 and 14.8 g kg^{-1} for maize, which were above the MRL established by the European Union and regulations of the Croatian Food Codex (Puntarić *et al.*, 2001). OTA concentrations in Albania were measured in wheat and maize samples. According to this investigation, OTA in the maize samples (139.2 and 8.58 μg kg^{-1}) exceeded the EU MRL, with values for commodities destined for human consumption set by the Albanian Food Codex (Shtëmbari and Topi, 2015). In Germany, an investigation of OTA occurrence in wheat in the years 1997 and 1998 (Birzele *et al.*, 2000) showed that the range of contamination was 0.6–0.7 μg kg^{-1} and the number of contaminated samples was 7/29. In Hungary, wheat flour OTA concentrations ranged from 0.11–0.15 in two positive samples out of 16, (Fazekas B *et al.*, 2002). In the Catalonian region of Spain, foodstuffs samples were collected from 12 cities and analyzed for OTA.

In breakfast cereals – only 2 out of 71 corn based samples were contaminated, with the mean positives of 0.73 μg kg^{-1}, while 7 out of 28 wheat and rice based samples were contaminated with OTA. None of the contaminated samples exceed the maximum tolerable limit of 3 μg kg^{-1} set by EU directives (Coronel *et al.*, 2012). In wheat bread samples in Portugal, the OTA content reached maximums of 0.49 μg kg^{-1} in the the region of Algarve and 0.43 μg kg^{-1} in the region of Bragança (Bento *et al.*, 2009). The ochratoxin A contamination rate in 100 wheat flour samples in Turkey was 87%, and none of these samples exceeded the maximum limits allowed by the Turkish Food Codex and European commission directives (Büyükünal and Vural, 2007). In another study on ochratoxin A in Turkey, 132 samples wheat flour, wholemeal flour, maize flour, wheat bread, wholemeal bread and maize bread, were collected between February 2005 and May 2006. The highest mean concentration was detected in a wholemeal flour sample with 9.30 μg kg^{-1} OTA. OTA was detected in 110, or 83% of samples and in 92 (70%) of all samples, levels were above the MRL set by Turkish and EU codices. These results shows that flour and bread may pose a risk for human consumption (Cengiz *et al.*, 2007). However, in another investigation in Turkey (Kara *et al.*, 2015) on cereal flour, all samples contained less OTA than the maximum limit set by the EU. In Pakistan, a study performed on the occurrence of OTA in breakfast cereals showed that 113/237 or 48%, of samples were found to be contaminated with OTA, and its concentration in 70/237 or 30% of the samples was above the EU maximum limit (3 μg kg^{-1}). The mean ± SD (μg kg^{-1}) was 2.22 ± 0.87 μg kg^{-1} and LOD range was 8.45 μg kg^{-1} (Iqbal *et al.*, 2014). In study on what samples collected in Iran by (Beheshti *et al.*, 2013), OTA was found in 17.5% of wheat flour samples, in the concentration below the limit set by the EU and Iran. In Egypt, (Abd Alla, 1996) OTA was not found in wheat flour but the highest level of OTA (up to 80.0 μg kg^{-1}) was found in yellow corn, 52.8% of contaminated sample. In Taiwan, a study was on OTA in cereals and their derivatives, was performed on 114 samples (75 were rice, and 39 wheat flour) collected from 2002-2004, OTA was detected in only two wheat flour samples ranging from 0.1 to 0.5 μg kg^{-1} (Lan-Chi Lin *et al.*, 2005). In Japan, between 2004 and 2007, foods have been examined for the presence of OTA. From a total of 1378 samples, 27 different foodstuffs were positive for OTA. Out of 160 of wheat flour, 79 samples were contaminated with OTA and similar proportion (about 50%) of OTA positive rye flour samples was found 18/40, but none of the samples were above the limit set by the EU (Aoyama *et al.*, 2010). The OTA contamination levels of wheat samples collected in Jordan (Nida & Ahmad, 2010) were below the maximum tolerable limit of ochratoxin A set by the European Union Commission regulation, the frequency of OTA positive samples was 29% (5 out of 17 samples) with a mean level of 2.26 μg kg^{-1}.

In the USA, a study of the occurrence of OTA in wheat, oat and barley-based breakfast cereals was performed, and none of the contaminated wheat samples exceeded the EU limit (Lee and Ryu, 2015). The Canadian study on the presence of ochratoxin A in a variety of grain-based and non-grain based foods in the Canadian retail market was performed from 2009 until 2014. Almost the half of the samples (3200 or 47%) did not contain OTA, while in the remaining 3657 samples OTA concentration ranged from 0.04 to 6.37 μg kg^{-1}. In maize products, 144/463 samples or 31% contained OTA, and exceeding EU regulations for MRL in 2 of them. OTA contamination of wheat products was much higher (425/583 or 73%), 14 samples being above the limit set by EU regulations (Kolakowski *et al.*, 2016).

Conclusion

The results of this survey indicated very low levels of OTA in flour samples with none of contaminated samples exceeding the EU limits. The results of this study indicate that the incidence of OTA contamination in wheat flour, maize flour and rye flour samples occurred mostly in the Prishtina municipality. This region also experiences higher temperatures and humidity levels than other regions of Kosovo. Considering these results, it could be concluded that OTA incidence in flour samples, which consisted of domestic and imported samples does not appear to be a serious public health problem for consumers of the Prishtina region.

Therefore, it can be concluded that our results may provide an idea about OTA contamination profiles in these flour samples in Kosovo. This survey was a first step to assure consumer protection against the risk of ochratoxin A exposure. Since there has not been enough research in Kosovo about the presence of OTA in cereals and derived products, more studies are required.

Ultimately, surveillance should be continuous and, widespread, and needs continuous monitoring and evaluation by state agencies.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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