Occurrence of citrinin in wheat cultivated in Kosovo and Albania during 2021

*A. Haziri, E. Emiri Sallaku, M. Zadravec, N. Kudumija, B. Berisha, M. Tafaj, S. Muji, E. Delia, F. Faiku, H. Ibrahimi and J. Pleadin**

Abstract

Citrinin (CIT) is a mycotoxin responsible for the contamination of many agricultural products, like wheat, barley, corn, rice and their products, as also other foodstuffs and feedstuffs used in human and animal nutrition. It is essentially produced by *Penicillium citrinum*, although it can also be biosynthesised from *Penicillium expansum* and *Penicillium verrucosum* and some species of *Aspergillus* and *Monascus*. However, several studies have shown that CIT is known for its genotoxic, hepatotoxic, fetotoxic and teratogenic properties. The aim of this study is to investigate the occurrence of CIT in wheat grain cultivated in Kosovo and Albania. Given the fact that wheat flour is the most consumed product in Kosovo and Albania, it is necessary to analyse the CIT in wheat in these two countries. In total, 60 wheat samples were tested from Fusha e Kosovës (Kosovo), Myzeqeja (Albania) and Fusha e Maliqit (Albania), as places with the highest wheat production. The enzyme-linked immunosorbent assay (ELISA) method was used to determine CIT concentrations. To identify moulds representing potential producers of CIT, traditional macroscopic and microscopic methods and the molecular PCR method of identification were implemented. CIT was detected in 96.6% and 86.6% of wheat grain samples collected in Kosovo and Albania, respectively. The maximum amount of CIT detected in wheat grain was 53.12 μg/kg in Kosovo, and 45.74 μg/kg in Albania. The amount of CIT found in wheat grain is not comparable with the maximal limits (MLs), as the European legislation does not provide limits for this mycotoxin. However, since there is generally a lack of data about CIT in cereals in Kosovo and Albania, the results can serve as an indicator of wheat grain contamination in this part of the Balkan Peninsula.

Key words: *citrinin; wheat grains; contamination; human exposure; Kosovo; Albania*

Arben HAZIRI, PhD, Full Professor, Faculty of Natural Science, University of Prishtina, Prishtina, Kosovo; Enkelejda EMIRI-SALLAKU, PhD, Full Professor, Faculty of Agriculture and Environment, Agriculture University of Tirana, Tirana, Albania; Manuela ZADRAVEC, PhD, Senior Research Associate, Nina KUDUMIJA, PhD, Research Associate, Croatian Veterinary Institute, Zagreb, Croatia; Bajram BERISHA, PhD, Full Professor, Faculty of Agriculture and Veterinary Medicine, University of Prishtina, Prishtina, Kosovo; Myqerem TAFAJ, PhD, Full Professor, Faculty of Agriculture and Environment, Agriculture University of Tirana, Tirana, Albania; Skender MUJI, PhD, Full Professor, Faculty of Agriculture and Veterinary Medicine, University of Prishtina, Prishtina, Kosovo; Etleva DELIA, PhD, Full Professor, Faculty of Agriculture and Environment, Agriculture University of Tirana, Tirana, Albania; Fatmir FAIKU, PhD, Full Professor, Hamide IBRAHIMI, PhD, Research Assistant, Faculty of Natural Science, University of Prishtina, Prishtina, Kosovo; Jelka PLEADIN*, PhD, Full Professor, Scientific Advisor in Tenure, Croatian Veterinary Institute, Zagreb, Croatia, (Corresponding author, e-mail: pleadin@veinst.hr)

Introduction

Mycotoxins are a group of secondary metabolites produced by different fungi that can contaminate human food and animal feed (Kalayu et al., 2017). Citrinin (CIT) is a mycotoxin essentially produced by *Penicillium citrinum*, although it can also be biosynthesized from *Penicillium expansum* and *Penicillium verrucosum* and some species of *Aspergillus* and *Monascus* (Li et al., 2003). Several studies have shown that CIT is known for its genotoxic, hepatotoxic, fetotoxic and teratogenic properties (Liu et al., 2003; Amalaradjou and Venkitanarayanan, 2008; Flajs and Peraica, 2009; Qingqing et al., 2010). In addition, the most important toxic property of this mycotoxin is nephrotoxicity (Iwahashi et al., 2007). Toxicity studies have shown that this secondary metabolite is involved as a causative agent in Balkan nephropathy endemicity (BEN) and is associated with a higher frequency of urinary tract tumours (Cosyns, 2003; Fuchs and Peraica, 2005). In 1986, CIT was classified within the third group as a possible human carcinogen by the International Agency for Research on Cancer (IARC, 1986). Studies, especially in Belgium and Germany, have shown that CIT or its metabolite, dihydrocytrinone was found in almost 90% of human urine tests, which could result in chronic exposure of the human body to this mycotoxin (Kiebooms et al., 2016).

In terms of food safety, CIT has been found to be responsible for the contamination of certain agricultural products, such as wheat, barley, corn and rice and their products (Pleadin et al., 2016, 2018). In addition, CIT is found in other foods besides cereals, which are used in animal nutrition (Bragulat et al., 2008). There are few available studies worldwide regarding CIT levels in cereals. Devegowda et al. (1998) reported that approximately 25% of the cereals consumed worldwide are contaminated with the CIT mycotoxin. In general, the extent of contamination is expected to be higher especially when climatic conditions are favourable for mycotoxin contamination. The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM) concluded in 2012 that CIT in food and feed presents a possible risk to human and animal health (EFSA, 2012). The need to collect further data on the presence of CIT in food in Europe was emphasized, in order to assess the risk from CIT. Therefore, in 2015, EFSA issued a call for proposals on research on citrine in cereals and cereal-based products from different geographical regions in Europe. It is important to point out that the European Union has not yet prescribed a maximal limit (ML) of CIT in food for humans and animals.

Kosovo and Albania are countries that have favourable climatic and geographical conditions for the growth of toxigenic fungi and the production of mycotoxins (Camaj et al., 2018). The population of Kosovo and Albania consume large amounts of cereals and cereal-based foods. Indeed, large quantities of commercialised cereals in Kosovo and Albania have been imported and we have no information on their possible contamination with the CIT mycotoxin. Therefore, the aim of this study was to determine CIT levels in wheat grain produced in the main cultivation areas in Kosovo and Albania, and to identify moulds as potential producers of this mycotoxin.

Materials and Methods

Sampling and sample preparation

During 2021, a total of 60 wheat grain samples were collected in Kosovo (n = 30) and Albania (n = 30). Wheat grain

of the 2021 genus were sampled from agricultural fields situated in Fusha e Kosovës (Kosovo), Fusha e Myzeqesë (Albania) and Fusha e Maliqit (Albania) (Figure 1). In Kosovo, wheat was taken directly to the mills, as farmers immediately send the entire harvest of wheat to the mills. There are 30-40 mills in the region of Fusha e Kosovës (Kosovo). In Albania, samples are taken from farmers, as farmers in Albania store their products in barns used for wheat storage. Sampling and sample preparation were performed in line with ISO 6497:2002 (ISO 2002) and ISO 6498:1998 (ISO 1998), respectively.

The final sample for analysis was taken according to Commission Regulation (EC) No 152/2009, defined as not less than 500 g for oilseed samples. The prepared test portions were ground into a fine powder with a particle size of 1.0 mm using an analytical mill (Cylotec 1093, Tecator, Sweden), and then stored at 4°C until analysis.

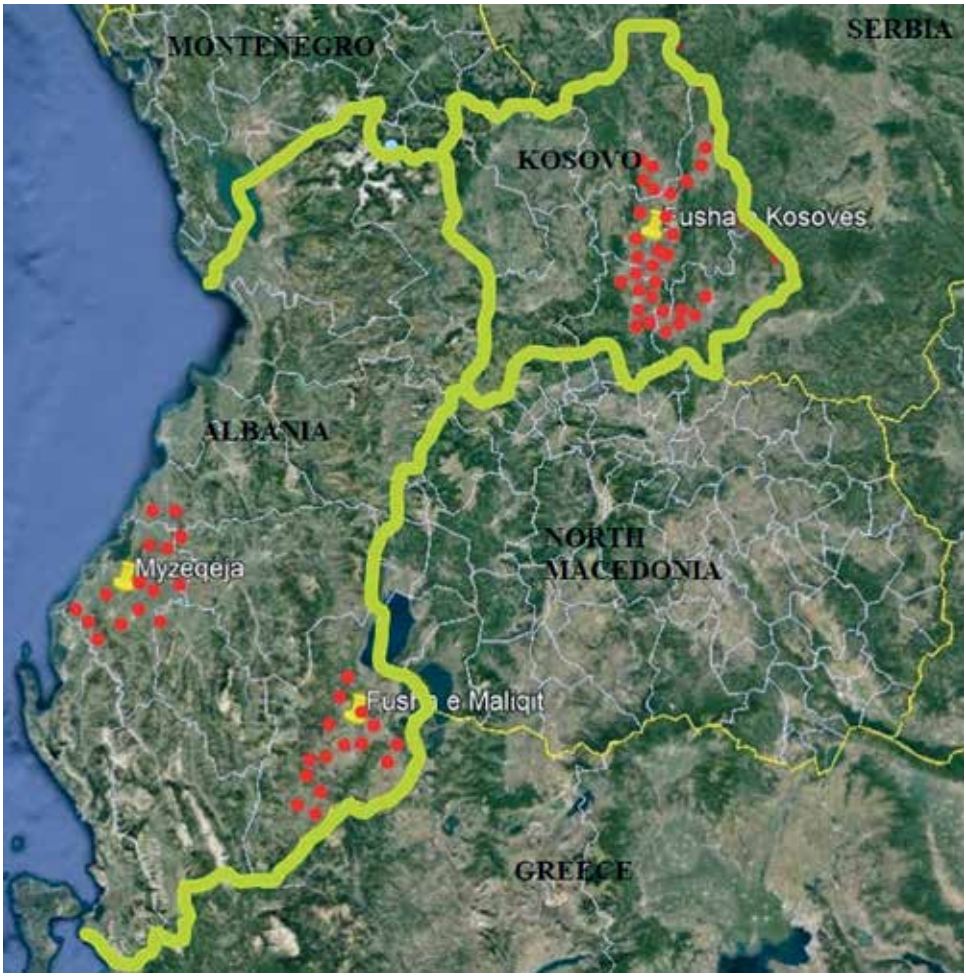

Figure 1. Sampling sites of wheat collected in Kosovo and Albania in 2021. The position of sampling is shown with red dots.

Mould identification

Moulds present on the surface of wheat grains were retrieved by surface disinfection by immersing grains for 2 min in a chlorine solution with 0.4% active chlorine, stirring occasionally, then draining the chlorine and rinsing with sterile deionised water. Up to 10 grains were transferring to 9-cm Petri dishes containing dichloran-glycerol agar base (DG-18, Merck, Germany). The inoculated agar media were incubated for seven days in darkness at 25±1 °C. Following this, macroscopic and microscopic characterisation of colonies was performed and they were identified to the genus level. For determination to the species level, the identified genera were sub-cultivated on malt extract agar (MEA, Difco International) and Czapek yeast extract agar (CYA, Difco International) and incubated for seven days at 25±1 °C in darkness.

Mould isolates were determined to the species level by defining their macroscopic and microscopic morphological characteristics. For the determination of micro-morphological characteristics, slides were prepared from the MEA medium, using lactophenol cotton blue (LPCB) as a mounting medium. Slides were analysed using differential interference contrast microscopy under oil immersion at 1000× magnification using an AX10 microscope (Zeiss, Germany). All isolates were identified according to Pitt and Hocking (2009) and Samson et al. (2019). Molecular identification of mould isolates was performed to verify the results of traditional identification methods and was carried out in full accordance with Lešić et al. (2021). The obtained sequences were aligned using Lasergene SeqManPro DNASTAR 13 (Madison, Wisconsin, USA). The edited sequences were compared to those available from the CBS-KNAW Fungal Biodiversity Centre database (http://www.cbs.knaw.nl) and the GeneBank using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Extraction and determination of CIT

After ginding of sample, to 5 g of homogenised sample, 70% methanol (12.5 mL) was added. Extraction was performed by vigorȯus 3-min shaking on a shaker, then filtration of the extracts through filter paper (Whatman, Black Ribbon). The supernatants were diluted with deionised water (1 + 1), and 50 μL was used for the ELISA test. A Ridascreen FAST CIT kit (Art. No. R6302) was used to perform the ELISA assay (R-Biopharm, Darmstadt, Germany). Each kit contains a micro-titre plate equipped with 48 wells coated with captured antibodies, five CIT standard solutions (0, 15, 45, 135 and 405 μg/L), conjugate (peroxidise), an anti-CIT antibody, the substrate/chromogen solution (tetramethylbenzidine) and the stop solution (1 N-sulphuric acid). All other chemicals used for the analysis were of analytical grade.

The ELISA test was performed using a ChemWell autoanalyzer (Awareness Technology Inc., Palm City, USA). The competitive ELISA assay was performed in line with the manufacturer's instructions. After adding the stop solution, absorbances were measured at 450 nm. In order to determine the concentration of CIT in each sample, the results obtained on a calibration curve were multiplied by the corresponding sample dilution factor. Final concentrations (μg/kg) were calculated based on the average recovery values obtained for each material.

Validation of the ELISA method

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated from the mean value of the analyses of 10 control samples constituted of a wheat grain plus 3- and 10-fold standard deviation, respectively. Trueness was determined using certified

reference material for flour (CRM) T17160QC (Food Analysis Performance Assessment Scheme, Fapas, Sand Hutton, York, UK), CIT thereby being certified in the range of 49.21-126.54 µg/kg. Recoveries were determined at three different levels (six replicates per concentration level per day) by spiking the control samples with CIT standard working solutions (500 µg/L) and used to the end of validation dependent on the level of fortification. In order to determine intermediate precision, the same steps were repeated under the same analytical conditions on two additional occasions within a 2-month period, using different lots of ELISA kits and chemicals.

Data analysis

Statistical analysis was performed using Statistica 10.0 Software (StatSoft Inc. 1984–2011, USA), with a statistical significance set at 95% (P=0.05).

Results and Discussion

In the Southeast European countries, the occurrence of CIT has been linked to Balkan endemic nephropathy (BEN) (Pfohl-Leszkowicz et al., 2002). As data are lacking on the presence of CIT in different countries, the European Commission called upon Member States to gather reliable data on year-to-year variations in its occurrence in order to be able to establish the MLs of CIT in different food and feed in the near future (EFSA, 2012). Although CIT studies in Southeast Europe have mainly been carried out parallel with the studies on ochratoxin A (Pepeljnjak et al., 2008; Milićević et al., 2009; Pleadin et al., 2012, 2013, 2016, 2018) and it is considered that there is a high likelihood of co-occurrence of these two mycotoxins, there is still a need for further collection of data on CIT in food and feed (Somorin et al., 2016). Hence, in,

Table 1. Identified mould species in wheat grain by country

Genus	Species	Kosovo	Albania
Aspergillus	*A. pseudoglaucus*	+	
	A. montevidensis	+	
	A. proliferans	+	
	A. Chevalieri	+	
	A. sajarovii	+	+
	A. tubingesis		+
	A. dimorphicus		+
	A. Amoneus		+
	A. candidus		+
Penicillium	*P. olsonii*		+
	P. brevicompactum	+	+
	P. cinamopurpureum	+	
	P. charlesii		+
	P. crustosum		+
	P. rubens		+
	P. griseofulvum		+
	P. auratiogriseum		+

this study CIT levels were determined in wheat grain sampled from agricultural fields, farms and mills in Kosovo and Albania, since data are lacking on the occurrence of CIT in wheat and wheat products in these two countries.

In order to identify moulds that are potential producers of CIT, traditional and molecular methods of identification were implemented. The results of identified species by country are shown in Table 1.

As presented in Table 1, none of the mould species known to be CIT producers were isolated in the analysed wheat samples. However, according to the Climate Monthly Bulletin (2021) the climate conditions for CIT producers, growth and production of CIT (high humidity and temperature over the range of 15-30 °C and optimally at 30 °C) were met. It is well known that the absence of a mycotoxin producer does not mean that the mycotoxin is not present in the samples, and vice-versa, i.e., the presence of a mycotoxin producer does not mean that the mycotoxin is present (Perdoncini et al., 2019; Daou et al., 2021). Absence of a CIT producer species is due likely to the presence of other more resistant moulds overgrowing CIT producers in the storage process, or the death of spores of CIT producers in unfavourable conditions.

In this study, 17 mould species specific for storage contamination were isolated: 9 *Aspergillus* species and 8 *Penicillium* species. All the isolated species prefer warm and dry conditions and represent storage contamination of wheat, especially teleomorph *Aspergillus* species (*A. montevidensis*, *A. proliferans*, *A. chevaliery*, *A. pseudoglaucus*, and the *Penicillium* species *P. brevicompactum* and *P. rubens* (Pitt and Hocking, 2009), unlike CIT producer mould species. An interesting observation obtained during the study showed that the mycoflora from Kosovo and Albania had a different composition. In Albania, the sample composition of moulds species is characterised by higher humidity and warmer conditions (more *Penicillium* species were isolated (5) than in Kosovo (2), and the presence of anamorph *Aspergillus* species: *A. tubingesis, A. dimorphicus, A. amoneus, A. candidus*). Conversely, in the Kosovo samples, only teleomorph *Aspergillus* species were isolated, and *Penicillium* species that prefer dry and hot conditions (Pitt and Hocking, 2009). Thus, it clearly shows that even small differences in climate can affect the mycoflora compositions.

Prior to the determination of CIT in the sampled materials, ELISA, as a quantitative screening method, was first validated and then applied for analyses. The validation results are shown in Table 2.

The validation of the ELISA method came up with a mean LOD of 15 μg/kg and a mean LOQ of 20 μg/kg. The mean value (n = 6) obtained in trueness determination equalled 91 μg/kg, which is in the range given by the CRM manufacturer. The mean recovery and the intermediate precision were determined to be 83.9% and 84.4%, respectively, with acceptable mean coefficients of variation of 7.5% and 11.7%, respectively (Table 2). Based on the obtained validation results, ELISA was recognised as a method suitable for the efficient determination of CIT in wheat grain.

The results on the presence of CIT in wheat grain, obtained within the frame of this study and displayed by country are presented in Table 3.

The mean concentration of CIT in wheat grain produced in Kosovo was 30.98 μg/kg, while in Albania 29.44 μg/kg. Among the cereals researched by Pleadin et al. (2016), the highest average concentration of CIT found in Croatia during 2014 was in wheat (92 ± 83 μg/kg). This is similar to the data recorded in 2015 by the same author (Pleadin et al., 2016), *i.e.*, 99 ± 102 μg/kg. The maximum CIT concentrations established in wheat samples in Croatia during 2014 and 2015 were 276 and 374 μg/kg, respectively

Table 2. Validation of the ELISA method used for CIT determination in wheat grain

Level of fortification (µg/kg)	Mean recovery (%)	Coefficients of variation [CV] (%)	Intermediate precision (%)	CV (%)
25	80.5	7.2	83.6	12.4
50	84.3	8.4	84.5	13.1
100	86.9	6.9	85.0	9.5

Table 3. The results of CIT determination in wheat grain by country

Country	N	Positives* (%)	Mean (µg/kg)	SD (µg/kg)	Min (µg/kg)	Max (µg/kg)
Kosovo	30	96.6	30.98	9.52	15.12	53.12
Albania	30	86.6	29.44	9.74	16.52	45.74

*Samples with CIT concentrations above the limit of detection (LOQ > 15 mg/kg).

(Pleadin et al., 2016). Just as in Croatia, the highest mean concentrations of CIT found in Bosnia & Herzegovina were those determined in wheat sampled both during 2014 (125 ± 112 µg/ kg) and during 2015 (124 ± 120 µg/kg) (Pleadin et al., 2016). Compared with the mean CIT concentrations found in Croatia (92 µg/kg in 2014 and 99 µg/kg in 2015) and Bosnia & Herzegovina (125 µg/kg in 2014 and 124 µg/kg in 2015), in this study a smaller quantity of CIT was observed in wheat samples taken from mills in Kosovo (30.98 µg/kg) and from small farmers of Albania (29.44 µg/kg).

In the EFSA study (2012), which included 30 samples of cereals and cereal-based products, CIT was detected in three samples in concentrations lower than the LOQ of the analytical method. The Panel on Contaminants in the Food Chain (the CONTAM Panel) concluded that these results do not represent a suitable basis for the assessments of CIT intake into the human body, since 27 of 30 analysed samples were not intended for human consumption. In comparison to these results, the present study found significantly lower concentrations of CIT, similar to the data obtained by other studies pooled by the EFSA, showing that the concentration of CIT in cereals may rise up to 420 µg/kg.

During 2021, CIT was detected in this study in 96% and 86% of wheat grain collected in Kosovo and Albania, respectively. These results indicate the highest number of positive samples with CIT compared to the results of Pleadin et al. (2016) for samples collected in Croatia (71%) and Bosnia and Hercegovina (66%). According to these results, the highest frequency and contaminated levels reported in the scientific literature are also those cereals samples from Russia (Kononenko and Burkin, 2008), Romania (Curtui et al., 1998), Bulgaria (Stoev et al., 2010), Tunisia (Zaied et al., 2012), Canada (Limay-Rios et al., 2017), Croatia (Culig et al., 2017) and Germany (Meister, 2004). In their analysis of cereals (n=766), Kononenko and Burkin (2008) detected CIT in 4.5% of wheat samples and 3.6% of barley samples in the concentration

range of 50-998 μg/kg, and in 1.9% of maize samples in concentrations of 218-953 μg/kg. Curtui et al. (1998) analysed 55 wheat and maize samples harvested in Romania and detected CIT in only one maize sample in a concentration of 580 μg/kg. Studies have also been carried out on animal feed samples originating from Romanian farms. All analysed samples contained CIT in the concentration range of 17-405 μg/kg, while in 25% of samples, CIT concentrations exceeded 405 μg/kg (Talmaciu et al., 2008).

Furthermore, in samples taken from pigs and chickens which were originating from Bulgarian farms during slaughtering, indicators of nephropathy were observed and attributed to the increased amounts of CIT determined in the samples. In 2006, CIT was detected in 92% of feed samples in the average concentration of 54.7 ± 27.5 μg/kg, while in 2007, 96% of samples were CIT-positive, with a mean CIT concentration of 120.5 ± 43.3 μg/kg. In a study in the United Kingdom on 141 cereal samples, 48 samples were contaminated with CIT in the highest concentration of 10 μg/kg (Scudamore and Hetmanski, 1995; Scudamore et al., 1997). In Tunisia, 200 samples of wheat were collected during 2010 and analysed for CIT contamination; the resulting incidence was 50%, with contamination levels ranging between 0.1 and 170 μg/kg, with an average of 28 μg/kg (Zaied et al., 2012). CIT was also evaluated in wheat samples (n = 37) from the Canadian Great Lakes Region between 2011 and 2014, with levels oscillating between <0.6 and 175.2 μg/kg (Limay-Rios et al., 2017). In dusts of stored wheat grains from a loamy region in central Belgium, CIT was found at higher levels of between 137.0 and 343.9 μg/kg (Tangni and Pussemier, 2005). Cereals (Culig et al., 2017) and cereal products (Meister, 2004) were also evaluated in Croatia and Germany, respectively. The levels found in wheat and maize from Croatia by Culig et al. (2017) varied according to the studied areas. In the Vukovar-Srijem region, the levels ranged between <1 and 103 μg/kg, with a mean level of 14.6 μg/kg, and in Osijek-Baranja area the range was <1-52.4 μg/kg, and the mean level 19.63 μg/kg (Culig et al., 2017). In Germany, 61.1% of the samples, including wheat samples, were contaminated, with levels ranging between <1-2.7 μg/kg (Meister, 2004).

CIT levels determined in the study area (Kosovo and Albania), did not differ in a statistically significant manner (P>0.05). Based on the report published by the Kosovo Agency of Statistics, KAS, (Series 2: Agriculture and Environment Statistics, 2021), it was observed that during 2021 all forms of atmospheric precipitation were present in Kosovo. The most significant rainfall is in the form of rain in the valleys and precipitation in the form of snow in the mountains. January 2021 had the maximum number of rainy days with 17 days in total, while August had only 4 days of rain. October 2021, the month of wheat sampling, had 13 rainy days. 2021 in Kosovo was a year with an average annual rainfall of 809.10 mm, which compared to other years had the highest annual rainfall after 2016 (851 mm). Therefore, 2021 in Kosovo was a year with relatively high humidity, compared to previous years. The average temperature inside the country fluctuated from +30 °C in summer, to -10 °C in winter. According to the data published by KAS, the maximum average temperature in 2021 was in August (31.0 °C), and the minimum average temperature was in February (-0.7 °C). In October 2021, the maximum temperature was 15.3 °C and the minimum was 4.4 °C.

From the data obtained from the Climate Monthly Bulletin (October 2021), it can be observed that the weather during October 2021 was different from the trends and expectations of recent months and years in Albania. This month had lower air temperatures relatively lower to the norm (-0.6 °C), and more precipitation (+6.8%), while rainy days above the threshold marked values

(+10.1%) against the multi-year average. October 2021 in Albania also had 13 days of rain, while the average rainfall for October 2021 in Albania was 135 mm. The maximum temperature in October was 30 °C and the minimum was 3.8 °C. Therefore, similar to Kosovo in terms of air temperature and humidity, Albania had a very warm and rainy climate in October 2021. These relatively high values of air temperature and humidity may be factors in the appearance of fungi *Aspergillus* and *Penicillium* in wheat grain collected in Kosovo and Albania, which are primarily responsible for the production of mycotoxins. Therefore, the approximate values of the air temperature and humidity of these two countries can be responsible for the similarity in CIT concentrations detected in wheat grain originating from Kosovo (30.98 μg/kg) and Albania (29.44 μg/kg).

Conclusion

The occurrence of CIT in wheat grain samples collected in 2021 in Kosovo and Albania was 96.6% and 86.6%, respectively. Despite the increase of positive samples with CIT, conclusions about the safety of these samples either in the form of food or feed cannot be reached until either maximum or the recommended CIT levels in food and feed are set by the legislation. However, he data provided by this study can serve as an indicator of CIT contamination of wheat grain originating from this part of Europe. Further studies are needed to more thoroughly investigate the conditions of CIT production, including storage conditions of wheat and the co-occurrence of CIT with ochratoxin A and other mycotoxins. Nonetheless, it should be kept in mind that other CIT sources are available for intake by food. Thus, a monitoring program should be conducted to periodically evaluate human exposure.

Acknowledgments

Funding was supported by the Ministry of Education, Science and Technology of the Republic of Kosovo and Ministry of Education and Sport of the Republic of Albania through the projects 2-905 and 577, respectively.

References

1. AGRICULTURE AND ENVIRONMENT STATISTICS (2021): Series 2, Water Statistics in Kosovo, Kosovo Agency of Statistics, https://ask.rks-gov.net/media/6724/statistikat-e-ujrave-ne-kosove-2020-2021.pdf.
2. AMALARADJOU, M. A. R. and VENKITANARAYANAN, K. (2008): Detection of Penicillium, Aspergillus and Alternaria species in fruits and vegetables. In: Barkai-Golan, R. and Paster, N. (eds): Mycotoxins in Fruits and Vegetables. Academic Press, Elsevier (225-249). 10.1016/B978-0-12-374126-4.00010-3
3. BRAGULAT, M. R., E. MARTINEZ, G. CASTELLA and F. J. CABANES (2008): Ochratoxin A and citrinin producing species of the genus Penicillium from feedstuffs. Int. J. Food Microbiol. 126, 43-48. 10.1016/j.ijfoodmicro.2008.04.034
4. CAMAJ, A., K. MAYER, B. BERISHA, T. ARBNESHI and A. HAZIRI (2018): Aflatoxin M1 contamination of raw cow's milk in five regions of Kosovo during 2016. Mycotoxin Res. 34, 205-209.10.1007/s12550-018-0315-4
5. CLIMATE MONTHLY BULLETIN (2021): Polytechnic University of Tirana, Department of Meteorology, Tirana, Albania, https://www.geo.edu.al/skedaret/buletini58.pdf.
6. COMMISSION REGULATION (EC) No 152/2009 laying down the methods of sampling and analysis for the official control of feed. Off. J. Eur. Union 2009, L 54, 1-130.
7. COSYNS, J. P. (2003): Aristolochic acid and Chinese herbs nephropathy: a review of the evidence to date. Drug Saf. 26, 33-48. 10.2165/00002018-200326010-00004
8. CULIG, B., M. BEVARDI, J. BOŠNIR, S. SERDAR, D. LASIC, A. RACZ, A. GALIC and Ž. KUHARIC (2017): Presence of CIT in grains and its possible health effects. Afr. J. Tradit. Complement. Altern. Med 14, 22-30. 10.21010/ajtcam.v14i3.3
9. CURTUI, V., E. USLEBER, R. DIETRICH, J. LEPSCHY and E. MÄRTLBAUER (1998): A survey on the occurrence of mycotoxins in wheat and maize from western Romania. Mycopathologia 143, 97-103. 10.1023/A:1006987205986

10. DAOU, R., K. JOUBRANE, R. G. MAROUN, L. R. KHABBAZ, A. ISMAIL and A. E. L. KHOURY (2021): Mycotoxins: Factors influencing production and control strategies. AIMS Agric. Food 6, 416-447. 10.3934/agrfood.2021025. 10.3934/agrfood.2021025
11. DEVEGOWDA, G., M. RAJU and H. SWANG (1998): Mycotoxins: novel solutions for their counteraction. Feedstuffs 70, 12-15.
12. EUROPEAN FOOD SAFETY AUTHORITY (2012): Scientific opinion on the risks for public and animal health related to the presence of citrinin in food and feed. EFSA panel on contaminants in the food chain (CONTAM). EFSA J. 10:1-81. 10.2903/j.efsa.2012.2605
13. FLAJS, D. and M. PERAICA (2009): Toxicological properties of citrinin. Arh. Hig. Rada. Toksikol. 60, 457-464. 10.2478/10004-1254-60-2009-1992
14. 14. FUCHS, R. and M. PERAICA (2005): Ochratoxin A in human kidney diseases. Food Addit. Contam. 1, 53-57. 10.1080/02652030500309368
15. 15. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (1986): Citrinin. Some naturally occurring and synthetic food components, furocoumarins and ultraviolet radiation. IARC Monogr. Eval. Carcinog. Risks Hum. 40, 67.
16. ISO 6497 (2002): Animal feeding stuffs - sampling. Technical Committee ISO/TC 34, Food Products, Subcommittee SC 10, Geneva.
17. ISO 6498 (1998): Animal feeding stuffs - preparation of test samples. European Committee for Standardization (CEN) Technical Committee TC 327, Geneva.
18. IWAHASHI, H., E. KITAGAWA, Y. SUZUKI, Y. UEDA, Y. H. ISHIZAWA, H. NOBUMASA, K. YOSHIHIDE, H. HIROSHI and I. YUMIKO (2007): Evaluation of toxicity of the mycotoxin citrinin using yeast ORF DNA microarray and oligo DNA microarray. BMC Genom 5, 8-95. 10.1186/1471-2164-8-95
19. KALAYU, Y. S., S. LING, Y. YANG, J. YUAN and S. WANG (2017): The preparation and identification of a monoclonal antibody against citrinin and the development of detection via indirect competitive ELISA. Toxins 9, 110. 10.3390/toxins9030110.
20. KIEBOOMS, J. A. L., B. HUYBRECHTS, C. THIRY, E. K. TANGNI and A. CALLEBAUT (2016): Quantitative UHPLC-MS/MS Method for citrinin and Ochratoxin A: Prevalence in Food, Feed and Red Yeast Rice Food Supplements. In: Toxins-Meeting Report Report from the 5th International Symposium on Mycotoxins and Toxigenic Moulds: Challenges and Perspectives (MYTOX) Held in Ghent, Belgium. 10.3920/WMJ2015.1971
21. KONONENKO, G. P. and A. A. BURKIN (2008): A survey on the occurrence of citrinin in feeds and their ingredients in Russia. Mycotox Res. 24, 3-6. 10.1007/BF02985263
22. LEŠIĆ, T., M. ZADRAVEC, N. ZDOLEC, A. VULIĆ, I. PERKOVIĆ, M. ŠKRIVANKO, N. KUDUMIJA, Ž. JAKOPOVIĆ and J. PLEADIN (2021): Mycobiota and Mycotoxin Contamination of Traditional and Industrial Dry-Fermented Sausage Kulen. Toxins 13, 798. 10.3390/toxins13110798. 10.3390/toxins13110798
23. LI, F., G. XU, Y. LI and Y. CHEN (2003): Study on the production of citrinin by Monascus strains used in food industry. Wei Sheng Yan Jiu 32, 602-605. 10.2520/myco1975.2003.Suppl3_185
24. LIMAY-RIOS, V., J. D. MILLER and A. W. SCHAAFSMA (2017): Occurrence of Penicillium verrucosum, ochratoxin A, ochratoxin B and citrinin in on-farm stored winter wheat from the Canadian Great Lakes Region. PLoS One 12, 1-22. 10.1371/journal.pone.0181239
25. LIU, B. H., F. Y. YU, T. S. WU, S. Y. LI, M. C. SU, M. C. WANG and M. SHIH (2003): Evaluation of genotoxic risk and oxidative DNA damage in mammalian cells exposed to mycotoxins, patulin and citrinin. Toxicol. Appl. Pharmacol. 191, 255-263. 10.1016/S0041-008X(03)00254-0
26. MEISTER, U. (2004): New method of CIT determination by HPLC after polyamide column clean-up. Eur. Food Res. Technol. 218, 394-399. 10.1007/s00217-003-0858-1
27. MILIĆEVIĆ, D., V. JURIĆ, D. VUKOVIĆ and M. MANDIĆ (2009): Natural occurrences of ochratoxicosis in slaughtered pigs from different regions of Serbia. Vet. World 2, 293-298. 10.3920/WMJ2008.1074
28. PEPELJNJAK, S., Z. CVETNIĆ and M. ŠEGVIĆ KLARIĆ (2008): Ochratoxin A and zearalenon: cereals and feed contamination in Croatia (1977-2007) and influence on animal and human health. Krmiva 50, 147-159.
29. PERDONCINI, M. R. F. G., M. J. SEREIA, F. H. P. SCOPEL et al. (2019): Growth of fungal cells and the production of mycotoxins. Cell growth. In: Vikas B. (Eds.) Cell Growth, IntechOpen, 10.5772/intechopen.86533.
30. PFOHL-LESZKOWICZ, A., T. PETKOVA-BOCHAROVA, I. N. CHERNOZEMSKY and M. CASTEGNARO (2002): Balkan endemic nephropathy and associated urinary tract tumours: a review on aetiological causes and the potential role of mycotoxins. Food Addit. Contam. 19, 282-302. 10.1080/02652030110079815
31. PITT, J. I. and A. D. HOCKING (2009): Fungi and food spoilage. New York: Springer. 10.1007/978-0-387-92207-2
32. PLEADIN, J., J. FRECE, N. KUDUMIJA, D. PETROVIC, V. VASILJ, M. ZADRAVEC, M. ŠKRIVANKO, I. PERKOVIĆ and K. MARKOV (2016): Citrinin in cereals and feedstuffs coming from Croatia and Bosnia and Hercegovina, Food Addit. Contam. Part B Surveill. 9, 268-274. 10.1080/19393210.2016.1210242
33. PLEADIN, J., M. ZADRAVEC, T. LEŠIĆ, J. FRECE, V. VASILJ and K. MARKOV (2020): Climate change - A potential threat for increasing occurrences of mycotoxins. Vet. stn. 51, 659-671. 10.46419/vs.51.6.8
34. PLEADIN, J., M. ZADRAVEC, T. LEŠIĆ, N. VAHČIĆ, J. FRECE, M. MITAK and K. MARKOV

(2018): Co-occurrence of ochratoxin A and citrinin in unprocessed cereals established during a three-year investigation period. Food Addit. Contam. Part B 11, 20-25. 10.1080/19393210.2017.1389994

35. PLEADIN, J., N. PERŠI, A. VULIĆ and M. ZADRAVEC (2012): Survey of mycotoxin feed contamination in Croatia. Biotechnol. Anim. Husb. 28, 167-177. 10.2298/BAH1202167P
36. PLEADIN, J., N. PERŠI, D. KOVAČEVIĆ, N. VAHČIĆ, G. SCORTICHINI and S. MILONE (2013): Ochratoxin A in traditional dry-cured meat products produced from subchronic exposed pigs. Food Addit. Contam. 30, 1827-1836. 10.1080/19440049.2013.825817
37. QINGQING, H., Y. LINBO, G. YUNQIAN and L. SHUQIANG (2010): Toxic effects of citrinin on the male reproductive system in mice. Exp. Toxicol. Pathol. 64, 465-469. 10.1016/j.etp.2010.10.015
38. SAMSON, R. A., J. HOUBRAKEN, U. THRANE, J. C. FRISVAD and B. ANDERSEN (2019): Food and Indoor Fungi, 2 ed., Westerdijk Fungal Biodiversity Institute: Utrecht, The Netherlands.
39. SCUDAMORE, K. A. and M. T. HETMANSKI (1995): Natural occurrence of mycotoxins and mycotoxigenic fungi in cereals in the United Kingdom. Food Addit. Contam. 12, 377-382. 10.1080/02652039509374318
40. SCUDAMORE, K. A., M. T. HETMANSKI, H. K. CHAN and S. COLLINS (1997): Occurrence of mycotoxins in raw ingredients used for animal feeding stuffs in the United Kingdom in 1992. Food Addit. Contam. 14, 157-173. 10.1080/02652039709374511
41. SOMORIN, Y., A. AKINYEMI, T. BERTUZZI and A. PIETRI (2016): Co-occurrence of aflatoxins, ochratoxin A and citrinin in "egusi" melon (Colocynthis citrullus L.) seeds consumed in Ireland and the United Kingdom. Food Addit. Contam. Part B. 9, 230-235. 10.1080/19393210.2016.1183051
42. STOEV, S. D., M. F. DUTTON, P. B. NJOBEH, J. S. MOSONIK and P. A. STEENKAMP (2010): Mycotoxic nephropathy in Bulgarian pigs and chickens: complex aetiology and similarity to Balkan endemic nephropathy. Food Addit. Contam. 27, 72x-88. 10.1080/02652030903207227
43. TALMACIU, E., I. SANDU and T. BANU (2008): Researches regarding the fungal contamination and the presence of citrinin in feed. Fungi Mycotox. 2, 212-217.
44. TANGNI, E. K. and L. PUSSEMIER (2005): Ochratoxin A and CIT loads in stored wheat grains: Impact of grain dust and possible prediction using ergosterol measurement. Food Addit. Contam. 23, 181-189. 10.1080/02652030500391911
45. ZAIED, C., N. ZOUAOUI, H. BACHA and S. ABID (2012): Natural occurrence of citrinin in Tunisian wheat grains. Food Control 28, 106-109. 10.1016/j.foodcont.2012.04.015

Pojavnost citrinina u zrnju pšenice uzgojenoj na Kosovu i u Albaniji tijekom 2021. godine

Dr. sc. Arben HAZIRI, redoviti profesor, Fakultet prirodnih znanosti, Sveučilište u Prištini, Priština, Kosovo; dr. sc. Enkelejda EMIRI-SALLAKU, redovita profesorica, Fakultet za poljoprivredu i okoliš, Poljoprivredno sveučilište u Tirani, Tirana, Albanija; dr. sc. Manuela ZADRAVEC, viša znanstvena suradnica, dr. sc. Nina KUDUMIJA, znanstvena suradnica, Hrvatski veterinarski institut, Zagreb, Hrvatska; dr. sc. Bajram BERISHA, redoviti profesor, Fakultet poljoprivrede i veterinarske medicine, Sveučilište u Prištini, Priština, Kosovo; dr. sc. Myqerem TAFAJ, redoviti profesor, Fakultet za poljoprivredu i okoliš, Poljoprivredno sveučilište u Tirani, Tirana, Albanija; dr. sc Skender MUJI, redoviti profesor, Fakultet poljoprivrede i veterinarske medicine, Sveučilište u Prištini, Priština, Kosovo; dr. sc. Etleva DELIA, redovita profesorica, Fakultet za poljoprivredu i okoliš, Poljoprivredno sveučilište u Tirani, Tirana, Albanija; dr. sc. Fatmir FAIKU, redoviti profesor, dr. sc. Hamide IBRAHIMI, asistent, Fakultet prirodnih znanosti, Sveučilište u Prištini, Priština, Kosovo; dr. sc. Jelka PLEADIN, redovita profesorica, znanstvena savjetnica u trajnom zvanju, Hrvatski veterinarski institut, Zagreb, Hrvatska

Citrinin (CIT) predstavlja mikotoksin za koji je utvrđeno da je odgovoran za kontaminaciju mnogih poljoprivrednih proizvoda poput: pšenice, ječma, kukuruza, riže i njihovih proizvoda, kao i drugih namirnica i hrane za životinje, osim onih na bazi žitarica, koje se koriste za prehranu ljudi i hranidbu životinja. Najviše ga proizvodi *Penicillium citrinum*, iako se može biosintetizirati i iz *Penicillium expansum* i *Penicillium verrucosum* te nekih vrsta *Aspergillus* i *Monascus*. Međutim, istraživanja pokazuju da su za CIT utvrđena genotoksična, hepatotoksična, fetotoksična i teratogena svojstva. Cilj ovoga istraživanja je bio utvrditi pojavnost CIT u zrnju pšenice koje se uzgaja na Kosovu i u Albaniji. S obzirom na činjenicu da je pšenično brašno najviše konzumirani proizvod na Kosovu i u Albaniji, analize CIT u zrnju pšenice u ove dvije zemlje od velikog su značenja. Ukupno je uzorkovano 60 uzoraka zrna pšenice na području poznatom kao Fusha e Kosovës (Kosovo), Myzeqeja (Albanija) i Fusha e Maliqit (Albanija), koja predstavljaju lokalitete na kojima se proizvodi najveća količina pšenice. Za određivanje koncentracije CIT korištena je imunoenzimna metoda ELISA. Za identifikaciju plijesni koje predstavljaju potencijalne producente CIT primijenjena je tradicionalna makroskopska i mikroskopska metoda te molekularna PCR metoda identifikacije. CIT je određen u 96,6 % i 86,6 % uzoraka zrnja pšenice prikupljenih na Kosovu i u Albaniji. Najveća količina CIT-a u zrnju pšenice proizvedenom na Kosovu bila je 53,12 μg/kg, a u Albaniji 45,74 μg/kg. Količina CIT utvrđena u pšenici ne može se usporediti s najvećom dopuštenom količinom (NDK), jer njegova razina u europskom zakonodavstvu nije definirana. No budući da podatci o količinama CIT u žitaricama uzgojenim na Kosovu i u Albaniji nisu dostupni, dobiveni rezultati mogu poslužiti kao pokazatelj kontaminacije zrnja pšenice na ovom dijelu Balkanskog poluotoka.

Ključne riječi: *citrinin, zrnje pšenice, kontaminacija, ljudska izloženost, Kosovo, Albanija*