FOOD SAFETY SITUATION IN ALBANIA, MYCOTOXIN'S IMPLICATION

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ABSTRACT

Recently, the food safety issue has been of relevant importance, due to a number of chemical contaminants of anthropogenic activity origin as well as secondary metabolites, produced by a number of microorganisms, and associated with a number of human and animal outbreaks. Mycotoxins, a family of secondary metabolites, produced by moulds have shown adverse effects on humans, animals, as results of crops contamination, used for food and feed, resulting in enormous economic losses of these commodities forbidden to go to the market, and secondly due to the healthcare system dealing with effects of foodborne diseases. Mycotoxins are found in different raw agricultural commodities and especially in cereal commodities, such as wheat, corn, and barley. Food consumption data in Albania show that wheat and wheat products consist the basis of the staple food, and consequently exposure to human population from mycotoxins present in this group of food remain important task for governmental stakeholders. Mycotoxins may impose various acute and chronic health effects on humans and animals. The major classes of mycotoxins are aflatoxins (AFs), with four major aflatoxins; fusarium toxins produced by *Fusarium* species, including Zearalenon (ZEN), Deoxynivalenol (DON), Fumonisins (FBs); Alternaria toxins etc. In this study, we have analysed the mycotoxins contamination in wheat flour from retail market in Albania. Twenty random samples were taken from different market in Albania. The objective of this study was to assess risk of mycotoxins exposure posed to humans. The samples were analysed for the presence of main mycotoxins: AFB1, AFB2, AFG1, AFG2, DON, ZEN, FB1, FB2 and OTA contamination by LC-MS/MS. Data produced through the analysis of wheat flour, we found none samples contaminated over the maximum limits as defined by national and European legislations. According to obtained results it can be concluded that the risk of the presence of mycotoxins in wheat flour samples during investigated period is very low.

Keywords: Mycotoxins, wheat flour, Albania.

INTRODUCTION

As the world's population grows, the intensification and industrialization of agriculture and animal production to meet increasing demand for food creates both opportunities and challenges for food safety. Climate change is also predicted to impact food safety, where temperature changes modify food safety risks associated with food production, storage and distribution.

These challenges put greater responsibility on food producers and handlers to ensure food safety. Local incidents can quickly evolve into international emergencies due to the speed and range of product distribution. Serious foodborne disease outbreaks have occurred on every continent in the past decade, often amplified by globalized trade (WHO 2015).

The EU has one of the highest food safety standards in the world – largely thanks to the solid set of EU legislation in place, which ensures that food is safe for consumers. A key tool to ensure the cross-border follow of information to swiftly react when risks to public health are detected in the food chain is RASFF – the Rapid Alert System for Food and Feed. Created in 1979, RASFF enables information to be shared efficiently between its members (EU-28 national food safety authorities, Commission, EFSA, ESA, Norway, Liechtenstein, Iceland and Switzerland) and provides a round-the-clock service to ensure that urgent notifications are sent, received and responded to collectively and efficiently. Thanks to RASFF, many food safety risks had been averted before they could have been harmful to European consumers (RASFF web). Albanian exports some of its products to the EU, so it has been mentioned several times in the portal of RASFF, for high nivel of biocontaminants in food products.

Contamination of food and agricultural commodities by various types of toxigenic moulds (fungi) is a serious and a widely neglected problem. Regardless of decades of extensive research, mould infection still remains a challenging problem (Munkvold 2003). It has been estimated by FAO that worldwide approximately 25% of the crops get contaminated by moulds and are affected by mycotoxins (CAST 1989; Rice and Ross 1994).

Wheat is one on the most important cereal crop in terms of cultivated area, production, imported and consumption in Albania. Due to the abundant production and the main role of wheat and its flour products in the diet of humans and animals, they play a very important role in endangering human health in case of contamination with health-threatening factors. In the farm and the warehouse, wheat can be contaminated by different microorganisms, especially fungi (Saari *et al.,* 1968).

Mycotoxins are toxic substances naturally produced by moulds that may contaminate agricultural commodities by growing on them. Despite efforts to control fungal contamination, toxigenic fungi are everywhere in nature and they can contaminate a wide range of agricultural products due to mould infestation both before and after harvest wherever humidity and temperature are sufficient. Thousands of mycotoxins exist, but only a few present considerable food safety hazards. *Aspergillus*, *Fusarium,* and *Penicillium*, all known field fungi, are the natural fungal flora associated with foods. The most prominent mycotoxins are aflatoxins, deoxynivalenol (DON), zearalenone (ZEA), ochratoxin, fumonisins, and patulin (Gaag *et al*. 2003).

The major classes of mycotoxins are aflatoxins (AFs), mainly produced by *Aspergillus* fungi, especially *A. flavus* and *A. parasiticus*. The four major aflatoxins are called B_1 (AFB1), B_2 (AFB2), G_1 (AFG1) and G_2 (AFG2) based on their fluorescence under UV light (blue or green) and relative chromatographic mobility during thin-layer chromatography (Bennett and Klich 2003). The diseases caused by aflatoxin consumption are called aflatoxicoses. Acute aflatoxicosis may results in death; chronic aflatoxicosis results in cancer, immune suppression, and other "slow" pathological conditions (Hsieh 1988). AFB1 is the most known potential hepatocarcinogenic in mammals and it is classified by the International Agency of Research on Cancer (IARC) as Group 1 carcinogen (IARC 1993).

Ochratoxin A (OTA) is produced by *Aspergillus ochraceus* and *Penicillium verrucosum* species. Exposure to OTA has been associated with distinct renal diseases in humans, quite endemic in the Balkans (in Bulgaria and ex-Yugoslavia), referred to as Balkan Endemic Nephropathy (BEN) and Urinary Tract Tumors (UTT) (Stoev et al., 1989). OTA is a nephrotoxin, affecting all tested animal species, though effects in man have been difficult to

establish unequivocally. It is listed as a probable human carcinogen (Class 2B) (Olsen et al., 2006).

ZEN is an estrogenic mycotoxin that can be produced by several field fungi including *Fusarium graminearum* (*Gibberella zeae*), *F. culmorum, F. cerealis, F. equiseti* and *F. semitectum* (Bennett and Klich, 2003). The critical effects of ZEN result from its potent estrogenic activity, considered as a possible causative agent in the outbreaks of precocious pubertal changes in the young children. The International Agency for Research on Cancer (IARC) has categorized ZEN as a class 2A carcinogen (IARC, 1993).

Deoxynivalenol (DON) is a natural-occurring mycotoxin mainly produced by *Fusarium graminearum* (Kushiro, 2008). It is also known as vomitoxin due to his strong emetic effects after consumption, because it is transported into the brain, where it runs dopaminergic receptors. The emetic effects of this mycotoxin were firstly described in Japanese men consuming mouldy barley containing *Fusarium* fungi in 1972 (Ueno, 1988).

In order to protect public health from this hazard, Commission Regulation (EC) setting maximum levels (ML) for certain mycotoxins in foodstuffs (Table 1). Maximum levels should be set at a strict level which is reasonably achievable by following good agricultural, fishery and manufacturing practices and taking into account the risk related to the consumption of the food (European Commission, 2006).

Cereal grains contaminated with mycotoxins represent a public health problem due to the high toxicity of these substances and also because they remain partially stable during the industrial processes (Bullerman and Bianchini, 2007) (Giménez *et al.,* 2013). The milling of the wheat can minimize mycotoxin concentrations in the fraction used for human consumption as these toxins are redistributed mainly in the bran, which is predominately used for animal feed (Herrera *et al.* 2009) (Cheli et al. 2013).

Wheat and wheat products are the basis of the food in Albania, therefore the food and feed safety issue (especially wheat commodity), recently has taken an important focus. In Albania, there are a few studies concerning the occurrence of mycotoxins in wheat and wheat products.

This study aimed to determine the levels of aflatoxins (B1, B2, G1 and G2), ZEN, DON and FUMs in the wheat samples imported in Albania during 2016.

METHODOLOGY

Standards and chemicals

Mycotoxins standards (AFB1, AFB2, AFG1 AFG2, DON, ZEN, FB1 and FB2) were purchased from Romer (Union, Mo, USA). Standard solutions were prepared in acetonitrile. Acetonitrile, methanol, acetic acid (Sigma-Aldrich, Steinheim, Germany) and ammonium acetate (Merck, Darmstadt, Germany) were of p.a. or LC-MS grade purity. Deionized water was prepared using a Milli-Q system (Millipore, Bedford, MA, USA).

Sample collection

In this study 20 representative wheat flour samples, were collected directly from different market in Albania. The wheat flour samples were originated by Russia and Kosovo,

according to the accompanying certificate of the commodity. The sampling procedure was carried out according to Commission Regulation (EC) No 401/2006 (European Commission, 2006a).

Mycotoxin analysis

Simultaneous determination of mycotoxins (AFB1, AFB2, AFG1, AFG2, DON, ZEN, FB1 and FB2) were done by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Sample preparation and the determination of mycotoxins was based on analytical procedures described by Driehuis et al., 2008 as well as Lattanzio et al., 2011. An amount of 20 g grounded sample was shaken with 100 ml of acetonitrile-deionised water mixture (70:30) for 1-hour period using a linear shaker IKA HS 501 digital (IKA Labortechnik, Staufen, Germany). A volume of 4 ml pre-filtered extract was pipetted into a vial and further preceded under vacuum evaporation to dryness (Syncore Polyvap, Büchi, Flawil, Switzerland). The residue was reconstituted with 0.5 ml of methanol-deionised water mixture (80:20) and passed to analytical vials. Finally, 20 µl was injected into LC-MS/MS. The detection and quantification was performed with an UPLC system Acquity coupled to a triple-quadrupole mass spectrometer Xevo TQ MS equipped with electrospray ionization (ESI) interface (Waters, Milford, MA, USA). The chromatographic separation was performed at the column Zorbax Eclipse Plus C18 Rapid Resolution HD column (2.1 x 100 mm, 1.8 µm, Agilent). The mobile phase consisted of two components mixed in gradient mode. Component A was deionized water and component B was methanol, both containing 0.5% acetic acid and 0.25 mM ammonium acetate. For each mycotoxin, the precursor ion and two product ions (a quantifier and a qualifier ion) were tracked together with the limits of detection (LOD), limits of quantification (LOQ) and recoveries for each single mycotoxin. Data acquisition and processing was done by means of MassLynx program (Waters, Milford, MA, USA).

RESULTS AND DISCUSSION

The current legislation about mycotoxins prevailing in Albania came into force in 2010 and Amendment Annex at 19.02.2016**,** it is harmonized with European Union legislations Commission Regulation (EC) No 1881/2006 of 19 December 2006.

Table 1: Maximum levels for certain mycotoxins in foodstuffs

Foodstuffs		**Maximum levels (µg/kg)**	
2.1	**Aflatoxins**	**B1**	**Sum of B1, B2,G1 and G2**
2.1.6	**All cereals and all products derived from cereals, including processed cereal products, with the exception of foodstuffs listed in 2.1.7, 2.1.10 and 2.1.12**	**2,0**	**4,0**
2.2	**Ochratoxin A**		
2.2.1	**Unprocessed cereals**	**5,0**	
2.2.2	**All products derived from unprocessed cereals, including processed cereal products and cereals intended for direct human consumption with the exception of foodstuffs listed in 2.2.9 and 2.2.10**	**3,0**	
2.4	**Deoxynivalenol**		
2.4.2	**Unprocessed durum wheat and oats**	**1 750**	

2.4.4	**Cereals intended for direct human consumption, cereal flour (including maize flour, maize meal and maize grits (21)), bran as end product marketed for direct human consumption and germ, with the exception of foodstuffs listed in 2.4.7**	**750**
2.5	**Zearalenone**	
2.5.1	**Unprocessed cereals other than maize**	**100**
2.5.3	**Cereals intended for direct human consumption, cereal flour, bran as end product marketed for direct human consumption and germ, with the exception of foodstuffs listed in 2.5.4, 2.5.7 and 2.5.8**	**75**
2.6	**Fumonisins**	**Sum of B1 and B2**
2.6.1	**Unprocessed maize**	**2000**
2.6.2	**Maize flour, maize meal, maize grits, maize germ and refined maize oil**	**1000**

(European Commission 2006; Fletorja zyrtare REPUBLIKËS SË SHQIPËRISË 2016)

A considerable number of mycotoxins of economic importance have been discovered through the second half of the twentieth century. Their presence may impose high risk to food commodities. Exposure of human population to mycotoxins is associated with human mycotoxicoses affecting humans: such as liver cancer, alimentary toxic aleukia, Balkan endemic nephropathy (BEN); and animal mycotoxicoses (turkey-X disease from aflatoxin, porcine nephropathy from OTA, vulvovaginitis from ZON etc.
So it is important analysed the cereals and their derivatives for provided the food safety. The limit of detection for the studied mycotoxins are presented in the Table 2.

Table 2: Limit of Detection (LOD) for studied mycotoxins [µg/kg]

Code number	DON	ZEN	FB1	FB2	OTA	AFB1	AFB2	AFG1	AFG2
1 (S)	<50	<20	<20	<20	<10	<0.2	<0.2	<0.2	<0.2

Twenty samples of imported wheat commodity were analyzed for the presence of mycotoxins (AFB1, AFB2, AFG1, AFG2, DON, ZEA, FB1, FB2 and OTA). The obtained results for mycotoxins occurrence in imported wheat flour revealed that none of the twenty analysed samples were contaminated with examined mycotoxins, so do not exceeded the maximum limits defined by Albanian and European legislations.

CONCLUSIONS

Performed LC-MS/MS analysis showed that imported wheat flour in Albania, were not contaminated with investigated mycotoxins (AFB1, AFB2, AFG1, AFG2, DON, ZEN, FB1, FB2 and OTA), because neither of samples were over the limit of detection (LOD). From this experimental work, it can be concluded that the risk of the presence of mycotoxins in imported wheat samples during investigated period is low.

Considering all the problems that causing contaminated cereals with mycotoxins in human and animal health, it is recommended to make systematic analyses, because cereals (especially wheat flour) are the basic food of population in Albania. Being the basic food of

the population, increases the possibility of exceeding the daily limits that humans and animals should consume.

Governments should make food safety a public health priority, as they play a pivotal role in developing policies and regulatory frameworks, establishing and implementing effective food safety systems that ensure that food producers and suppliers along the whole food chain operate responsibly and supply safe food to consumers.

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