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OFFICIAL CONTROL OF WHEAT MYCOTOXINS CONTAMINATION IN THE SLOVAK REPUBLIC

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ABSTRACT

It is important for the protection of public health that maximum levels are set on unprocessed cereals in order to avoid, that highly contaminated cereals can enter the food chain and to encourage and ensure that all measures are taken during the field, harvest and storage stage of the production chain. The contamination of winter wheat grain by toxins with focus on the genus *Fusarium* was monitored within the years 2009 – 2011 under the official control according to EC Regulation 401/2006 and 178/2010 on the territory of the Slovak Republic. The concentration of deoxynivalenol (DON) and nivalenol was determined by HPLC/DAD detector and concentration of zearalenone (ZEA) by HPLC/FLD detector. Deoxynivalenol was the most common (dominant) Fusarium toxins in 2009-2011 with a concentration ranging from 20 $\mu g.kg^{-1}$ - 2 651.79 $\mu g.kg^{-1}$. 4 samples contained the content of deoxynivalenol which was over the EC Regulation no. 1881/2006 about setting the maximum levels for certain contaminants in foodstuff. Trichothecenes nivalenol occurred regularly together with deoxynivalenol. 12 % of wheat samples were contaminated with two toxins deoxynivalenol and zearalenone, 7 % of samples were analyzed for concurrent occurrence of zearalenone + deoxynivalenol + nivalenol.

Keywords: : mycotoxins, wheat, Slovak republic

INTRODUCTION

Food product quality is a complex concept involving many aspects; among them and foremost is public or consumer health. Over the last few years, food safety attention worldwide has been increasingly focused on finished goods and line production. This attention is particularly applied to the cereal sector because wheat and its products, bread and pasta, are basic foods. Cereals and other crops are susceptible to attacks of various genera of fungi, which are ubiquitous and widespread at all levels of the food chain, many of which produce toxic metabolites, so-called mycotoxins. These may affect the sensory quality and safety of human foods and animal feeds **(Abbas *et al.*, 2002)**. It is important for the protection of public health that maximum levels are set on unprocessed cereals in order to avoid that highly contaminated cereals can enter the food chain and to encourage and ensure that all measures are taken during the field, harvest and storage stage of the production chain (by applying good agricultural, harvest and storage practices) **(EC 856/2005, 2005)**.
In conditions of Central Europe fusariosis occur annually at 70% of cereal crops. Attacks and economically significant crop losses occur about every third year **(Wakulinski, 1990)**. This claim was also confirmed in past by **Šrobarova** and **Vaškova (1987)**, in their experiments were isolated in Slovakia most often species: *F. culmorum*, *F. graminearum*, *F. avenaceum*, *F. oxysporum*, *F. moniliforme* and *M. nivale*. Current range of Fusarium species in winter cereals is similar, it is changed only the order of their dominance and frequency: *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. Poae* and *M. nivale* **(Hudec, 2006)**.
All *Fusarium* species that infect cereals can produce one or more mycotoxins. Many of the *Fusarium* species that infect cereals, produce trichothecene mycotoxins. These mycotoxins are divided, based on their structure, into type A and type B trichothecenes. The most common trichothecene found in cereals is deoxynivalenol (DON), which is type B trichothecene that is produced predominantly by *Fusarium graminearum* and *F. culmorum*. Isolates of these species are either DON or nivalenol producers **(Miller *et al.*, 2001)**.
Zearalenone (ZEA) (previously known as F-2 toxin) is a nonsteroidal oestrogenic mycotoxin biosynthesized by a variety of Fusarium fungi, including *F. graminearum* (*Gibberella zeae*), *F. culmorum, F. cerealis, F. equiseti, F. crookwellense* and *F. semitectum*, which are common soil fungi, in temperate and warm countries, and are regular contaminants of cereal crops worldwide **(Bennett, Klich, 2003)**.
The aim of this work was to evaluate wheat mycotoxin contamination in the Slovak republic especially for deoxynivalenol, zearalenone and nivalenol and their co-occurence.

MATERIAL AND METHODS

The contamination of winter wheat grain by toxins with focus on the genus *Fusarium* were monitored within the years 2009 – 2011 under the official control according to EC Regulation 401/2006 and 178/2010 on the territory of the Slovak Republic. The concentration of mycotoxins were determined by HPLC method. In total 189 samples were analyzed for mycotoxin determination. Samples were extacted and evaluated according to methodologies of the State Veterinary and Food Administration of the Slovak republic **(SVPS 2013)**.

Zearalenone detemination method

HPLC/FLD system with RP column LC-18, 250 mm x 4.6 mm was used as analytica column. The mobile phase consisted of ACN:H_2O: Acetic acid (51:47:2 v/v). All separations were carried out at 30°C applying a flow rate of 1 $ml.min^{-1}$. The injection volume was 50 µl. Ochratoxin A was detected with fluorescence detector at 333/444 nm. Zearalenone was detected with fluorescence detector at 274/455 nm.

Deoxynivalenol detemination method

HPLC/DAD system with RP column C-18 (150 mm x 4.6 mm 5µm) was used as analytica column endcapped (Gemini,Phenomenex 150 mm x 4.6 mm 5µm). The mobile phase consisted of H_2O : Acetonitrile : methanol (90/5/5 v/v/v). All separations were carried out at 40°C applying a flow rate of 1 $ml.min^{-1}$. The injection volume was 50 µl. Deoxynivalenol was detected with DAD detector at 218 nm.

Nivalenol detemination method

HPLC/DAD system with RP endcapped column C-18, (Gemini, Phenomenex 150 mm x 4.6 mm 5µm) was used as analytica column. The mobile phase consisted of H_2O : metanol : acetonitrile (90/5/5 v/v/v). All separations were carried out at 40°C in column, sample temperature 10°C applying a flow rate of 1 $ml.min^{-1}$. The injection volume was 50 µl. Nivalenol was detected with DAD detector at 218 nm with time of analysis 20 min.

Since the distribution of mycotoxins in granis are not homogeneous, samples were prepared, and especially homogenised with special concern. Each laboratory sample was in the laboratory milled and thoroughly mixed according to the procedure established and demonstrated to achieve complete homogenisation. Methods of analysis used for food control were in accordance with the provisions of items 1 and 2 of Annex III of Regulation (EC) no. 882/2004.

Laboratory complyes the provisions of Article 12 of Regulation (EC) no.882/2004 for official controls performed to ensure the verification of compliance the feed and food law, animal health and animal welfare.

RESULTS AND DISCUSSION

Food are not placed on the market if it contain a contaminant listed in the Annex of Commission Regulation (EC) No. 1881/2006 setting maximum levels of certain contaminants in foodstuffs. The maximum content listed in the Annex is applied on the edible part of the foodstuffs, if in Annex is not specified otherwise. Collected and subsequently analysed samples of cereals were evaluated in terms of maximum levels of certain mycotoxins in accordance with Annex of EC no. 1881/2006.

Trichothecene deoxynivalenol (DON) is probably the best known and most common mycotoxin contaminant of food and feed grains. In fact it occurs anywhere in the world where are grown cereals **(Chu, 1977)**. Summarized data from EU by contamination of wheat by mycotoxins indicates that a number of positive findings are 14% for nivalenol (NIV) and 61% for DON **(EU, 2003)**.

Based on previously published information, DON is hygienically and economically most serious mycotoxin. As is reported by **Prugar, Bjelková, (2008)**, as well as on the basis of our results obtained in three years 2009 – 2011, it should be pointed out, that particularly DON could be regarded as an indicator of the overall mycotoxin contamination.

Number of analyzed samples for mycotoxin determination are shown in Table 1. DON was determined in 85 % samples but only in 4 samples the content of DON exceeded the EC no. 1881/2006 about setting the maximum levels for certain contaminants in foodstuff, what represent only 0.02 % analyzed samples. Zaralenone (ZEA) was detected in 65 % samples and nivalenol (NIV) in 63% samples. In both cases concentration of ZEA and NIV did not exceeded legislation limit.

ZEA concentration in wheat grain showed the same trend of incidence in each year as DON, although its concentration was lower, fluctuating between 1 to 288.0 $µg.kg^{-1}$. The reason is probably the fact that ZEA producers are the same species of *Fusarium*, which are *F. graminearum, F. culmorum.* Besides these, the producers of this toxin are also *F. equiseti* a *F. cerealis* **(Bottalico, Perrone, 2002)**.

Table 1 Overview of the analyzed mycotoxins in wheat (2009-2011)

Mycotoxin	No. of samples	LOD ($µg.kg^{-1}$)	under LOD	over LOD	MRL	No. over MRL
DON	189	20	28	161	1250*	4
ZEA	175	1	62	113	100	0
NIV	80	20	30	50	0	0

Legend: LOD - limit of detection, MRL - maximum residue limit, EC No 1881/2006 about maximum levels of certain contaminants in foodstuffs; * 1250 $µg.kg^{-1}$ is limit for unprocessed cereals other than durum wheat, oat and maize, MRL of unprocessed durum wheat, oat and maize is 1750 $µg.kg^{-1}$.

Summarized data from EU contamination of wheat by NIV indicates that a number of positive findings represent 14% (EU, 2003). Results of collected samples from SR, about wheat contamination by NIV follows that positive findings and represented occurrence of 60.46 %, 7.69 % and 30.43 % in 2009, 2010 and 2011.

EC is considering whether to set a separate limit for NIV mycotoxin. The EC does not have yet sufficient database of measured levels of NIV in European cereals and SR also did not have this database.

Detection of fungal contaminants in food is therefore an important step to confirm the safety and high quality of food. During storage of cereal grains, the growth of fungi and mycotoxins production, is a result of combination of several factors, such as deterioration of plant material, temperature, gas (O_2 and CO_2), moisture, inoculum size, storage period and the incidence of other microorganisms (**Abramson 1998; FAO 2001)**.

In our study, wheat contamination by DON was evaluated for three regions of Slovakia separately. The contamination by the mycotoxins in different Slovak regions were statistically significantly different. The highest wheat contamination by DON was detected in east Slovakia. The average level for the east was 140.58 $µg.kg^{-1}$, central Slovakia 98.541 $µg.kg^{-1}$ and west 72.754 $µg.kg^{-1}$ of DON concentration. In west Slovakia the highest share of wheat production in SR is achieved. In this region was determined the lowest DON contamination. As it was presented by **Hudec (2009)**, Fusarium head blight in 2009 was a disease, which perhaps the most followed the regional differences. This disease is characterized by high harmfulness and difficult protection, but it is very sensitive on time of infection - stage of flowering. During flowering of winter wheat, at most areas of the Slovakia was dry and warm weather, which ultimately led to Fusarium head blight negligible significance and low incidence in 2009. Fusarium head blight had only on a few places of Slovakia higher occurrence, but mostly unimportant.

Based on the obtained results it can be concluded that in west region of SR were recorded higher levels of trichothecene DON in 2010 and 2011 compared to other regions of the Slovakia (central and east). In the year 2009 the highes average levels of mycotoxins were recorded at the east of SR (slightly higher than on the west of SR), which can be explained by higher rainfall in 2009 during the flowering of the cereals in this area, as well as regional disparities **(Hudec 2009)**.

Higher content of mycotoxins in the west of SR in years 2010 and 2011 confirm the theory of higher incidence of mycotoxins, especially in more humid areas or in years with rainy weather **(Vaňová *et al.*, 2000)**. Area of the greatest risk of wheat mycotoxins accumulation within the period 2009 - 2011 was west of Slovakian region.

In all evaluated years, the average content of trichothecene DON was significantly affected by year of cultivation. In Table 2 is the overview of samples analyzed for DON contamination by year. Overall, concentrations of DON in wheat samples in year 2009 are considered as low, compared to the years 2010 and 2011. The reason of this finding is probably a low incidence of rainfall during the flowering of the cereals in the year 2009.

Table 2 Summary of samples analyzed for deoxynivalenol contamination by year.

Year	No of positive samples / total number of samples	Mean ($µg.kg^{-1}$)	Median ($µg.kg^{-1}$)	Concentration range ($µg.kg^{-1}$)
2009	55/70	144,77	56,25	20 – 2 220
2010	70/77	481,23	343	20 – 2 438,22
2011	36/42	300,16	97,47	20 – 2 651,79

In 2009, 70 samples were analyzed. Out of this, one sample did not comply the requirements of legislation, and maximum content of DON was exceeded. It was 9 500 kg of winter wheat, variety Pegassos with DON content of 2220 $µg.kg^{-1}$, which was collected in a primary producer of cereals in west SR. The maximum allowable level is 1 250 $µg.kg^{-1}$.

In year 2010, 77 samples were collected and two samples did not meet the requirements of the legislation. In a sample of winter wheat durum, variety Pentadur was detected 2438 $µg.kg^{-1}$ of DON, and the maximum allowed content is 1750 $µg.kg^{-1}$ for durum wheat. Total quantity of this sample was 275 000 kg. The second sample was winter wheat, variety Bona Dea, in which 1833 $µg.kg^{-1}$ of DON was detected, but within the bounds of law (EC 1881/2006) and uncertainty of the sample was evaluated as appropriate. Both samples were from west SR. In year 2010 was found one unsatisfactory sample of whole grain flour with DON content of 938 $µg.kg^{-1}$. The maximum allowed content is 750 $µg.kg^{-1}$.

42 samples were collected in year 2011, and one sample did not meet the requirements of the legislation. In a sample of winter wheat durum, variety Pentadur was detect 2560 $µg.kg^{-1}$ of DON. Total quantity of this sample was 275 000 kg.

The co-occurrence of some Fusarium mycotoxins in cereals

High attention is payed to the toxic effects of simultaneous exposure of multiple mycotoxins to animals. **Speijers *et al.* (2004)** and **Gajęcki *et al.* (2007)** presented that concern of synergistic toxic effects was in many cases confirmed. For this reason, some mycotoxins are currently the subject of food legislation, but also the subject of studies concerning their common occurrence in the primary products of plant origin. Results of numerous studies indicate that the extent of the risk of contamination of feed / food by several mycotoxins depends on the level and type of mycotoxins, species, age of animals / people and their health, organ that is

assessed and interactions of mycotoxins can be complementary, synergistic or antagonist **(Huff *et al*., 1988; Diaz *et al*., 1994; Boeira *et al*., 2000; Ledoux *et al*., 2003; Speijers and Speijers, 2004; Jestoi, 2005; Malekinejad *et al*., 2007; Njobeh *et al*., 2010)**.

On the analyzed samples, it is possible to demonstrate, that in many cases, as shown in table 3, is the synergistic incidence of several mycotoxins by Fusarium on cereals collected in SR. Trichothecene NIV occur regularly together with DON. For wheat, it was 15.5% of positive samples. Based on our results we can conclude that the wheat is a crop where can be expected parallel occurrence of DON and NIV. **SCOOP** presented **(2003)** that trichothecene NIV occurred regularly together with DON worldwide. This highlight that it is reliable to control and assume the occurrence of NIV, depending on the occurrence of DON in the sample.

Stehlíková (2007) presented results that in contaminated grain by *Fusarium*, it can be very often observed co-contamination of zeralenone and other trichothecene toxins. The concurrent occurrence of ZEA with other trichothecene toxins can be seen in Table 3. 12 % of wheat samples were contaminated by two toxins DON and ZEA, 7 % of samples were positive analyzed for concurrent occurrence of ZEA + DON + NIV.

Table 3 The co-occurrence of mycotoxins in wheat samples analyzed in 2009-2011

Mycotoxin / mycotoxins	Positive samples
DON	40 (15,5%)
DON + NIV	40 (15,5%)
DON + ZEA	31 (12,01%)
DON + ZEA + NIV	17 (6,59%)

CONCLUSION

From all 189 analyzed wheat samples, only in 4 samples the content of deoxynivalenol was over the EC Regulation no. 1881/2006, setting the maximum levels for certain contaminants in foodstuffs. Deoxynivalenol was the most common (dominant) Fusarium toxin in 2009-2011 with the concentration ranging from 20 $\mu g.kg^{-1}$ to 2 651.79 $\mu g.kg^{-1}$. Deoxynivalenol was detected in 85 % of samples. Zearalenone was detected in 65 % and nivalenol in 63 % of samples. The concentrations of zearalenone and nivalenol did not exceeded the legislation limit. Fusarium contamination of crops may contain one or more trichothecenes / mycotoxins. Trichotecene nivalenol occured regularly together with deoxynivalenol. 12% of analyzed samples were contaminated by two toxins deoxynivalenol and zearalenone, and 7 % of samples were analzyed for co-occurrence of zearalenone + deoxynivalenol + nivalenol. Within analyzed samples from three regions of Slovakia (west, central, east), significant differences were found in the incidence of deoxynivalenol, probably depending on weather conditions during flowering of winter wheat. Overall, concentrations of deoxynivalenol in wheat samples in year 2009 are considered as low, compared to the years 2010 and 2011. Content of deoxynivalenol was highly significantly affected by year of cultivation. Co-occurrence of mycotoxins in wheat (DON + NIV) was confirmed.

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