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PRELIMINARY RESULTS ON DEOXYNIVALENOL DEGRADATION IN MAIZE BY UVA AND UVC IRRADIATION*

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Summary: *The aim of this work was to investigate the possibility of using UV irradiation for degradation of deoxynivalenol (DON) in naturally contaminated maize samples. The study was carried out by varying the distance of the contaminated maize from the UVA (368 nm) and UVC (254 nm) light source and duration of exposure of contaminated maize to UV irradiation. Two control samples of maize were used for the irradiation procedure, at DON levels of 1.902 mg/kg and 5.334 mg/kg. The samples were exposed to both UVA and UVC light at two different distances from UV lamp (15 and 30 cm) during three exposure intervals (30, 60 and 120 minutes). After irradiation DON content was determinated so the reduction levels could be quantified. Generally, the results showed reduced DON content in treated samples, but they were not consistent. The most probable reason for this was a very uneven DON distribution in naturally contaminated samples. For this reason, further research must be performed and should include irradiation of artificially inoculated samples with consistent DON distribution, the amount of irradiated sample needs to be enlarged and the higher number of replicates should be analyzed. As an additional improvement, an increase in frequency of sample mixing during the irradiation procedure needs to be done.*

Key words: *degradation, deoxynivalenol, maize, ultra-violet irradiation.*

INTRODUCTION

Food contaminants, including mycotoxins, play important role in food chain. Deoxynivalenol (DON), as one of the most widely spread mycotoxin from the trichotecene group, is most frequently produced by *Fusarium graminearum* and *Fusarium culmorum* molds. Maize is one of the most sensitive cereals to the presence of these molds. A disease of maize caused by these molds is known as *Gibberella* ear rot (JECFA, 2001). Infection of maize may lead to a grain size and protein decreasing as well as a harmful effect on germination. The final result is a decrease in yield and feed quality. Also, this mycotoxin may lead to various health effects in animals, especially in pigs and poultry (Pestka, 2007). Primary chronic effects in animals include a decrease in food intake and growth, as well as altered immune function. The primary health problem is acute poisoning with high doses of DON because of its ability to cause acute gastroenteritis and vomiting (Pestka, 2007).

In order to decrease mycotoxin exposure to animals and humans, Juodeikiene et al. (2012) presented three possibilities to avoid the harmful effect of mycotoxin contamination of food and feed, which included: prevention of contamination, decontamination of mycotoxin-containing food and feed, and inhibition of mycotoxin absorption into the digestive tract. If mycotoxin contamination cannot be prevented, physical and chemical decontamination methods

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need to be employed (Jouany, 2007). Many techniques (ultraviolet irradiation, ozone gas, pulsed light technology, sunlight, γ-radiation, microwave heating, food processing, microbial detoxification, adsorbent materials, nixtamalization, extrusion, mechanical detoxification, etc.) were used for mycotoxin degradation (Garg et al., 2013; Giordano et al., 2012; Moreau et al., 2011; Herzallah et al., 2008; Bullerman and Bianchini, 2007; Binder and Binder, 2004; Avantaggiato et al., 2004; Elias-Orozco et al., 2002; House at al., 2003).

Ultraviolet (UV) irradiation as a physical method has been studied to destroy mycotoxins, especially aflatoxins, for many years (Yousef and Marth, 1986; Samarajeewa and Gamage, 1988; Garg et al.,2013; Diao et al., 2015). However, there were not enough reports on DON degradation using UV light until recently.

Murata et al. (2008) have demonstrated in vitro that UV irradiation is effective for reducing the levels of both solid and aqueous DON and zearalenone. The authors investigated the effects of mild and strong UV irradiation at wavelength of 254 nm. In the case of mild exposure to UV irradiation, DON and zearalenone levels (initial concentration of 30 mg/kg each) were reduced by increasing the time of exposure. After exposure of 60 minutes, the toxins were undetectable. By applying strong UVC irradiation, contents of these mycotoxins were more quickly reduced, but in the same time-dependent manner. Murata et al. (2008) suggested that DON is probably degraded due to disintegration of the mycotoxin structure into less toxic or nontoxic fragments, suggesting that UV irradiation could be useful for elimination of mycotoxin contamination in feedstuffs. Three years later, the same authors (Murata et al., 2011) examined the effect of exposure of maize silage containing DON to UV irradiation. DON was artificially added at level of 60 mg/kg in dried silage. The sample exposure to UV irradiation was performed by using UVC fluorescent lamp at a wavelength of 254 nm for 0, 15, 30 and 60 minutes. A statistically significant reduction of DON was observed at exposure time of 30 minutes, to 13 mg/kg (22%), and at the time of exposure of 30 minutes, to 12 mg/kg (21%). Besides these two experiments, there are no available trials about the effectiveness of UV irradiation on DON reduction.

The ultraviolet region of the light spectrum consists of a range of wavelengths between 200 and 400 nm. This region is divided into three types: UV-A (315-400 nm) which causes skin tanning, UV-B (280-315 nm) which can lead to skin cancer, and UV-C (200-280 nm) which has germicidal activity (Sastry et al., 2000). As can be concluded from the literature review, the possibility of DON degradation in maize is not sufficiently studied. Also, there are insufficient data on the impact of UVA and UVC radiation to DON content in maize.

The aim of this work was to explore the possibility of using UVA and UVC irradiation for degradation of DON in naturally contaminated maize samples. The study was carried out by varying the distance of the contaminated maize from the UVA and UVC irradiation source and duration of exposure of contaminated maize to UV irradiation.

MATERIAL AND METHODS

Samples and sample handling. Maze samples, used as the object for UV irradiation (and as control samples), were collected from random locations in Serbia. The samples, naturally contaminated with DON, were tested for DON content and then divided, according to their DON level, into two groups (≈2 mg/kg and ≈5 mg/kg). Samples from the same group were then merged and thoroughly mixed. Each group now represented one sample intended for treatment by UV irradiation procedure. Such samples were stored in a freezer at –20 °C until treatment. Prior to each treatment, the samples were allowed to reach room temperature.

Chemicals. Acetonitrile and water (all HPLC grade) were purchased from Sigma (St. Louis, MO, USA). DON standard solution was also purchased from Sigma as an analytical standard. Calibration solution was prepared in ethyl-acetate:methanol (19:1, v/v) at the concentration of 0.1 mg/ml from crystalline substance according to AOAC method 986.17. Stock solution was prepared by measuring 1.00 ml of calibration solution of DON into a 10 ml volumetric flask and diluting to volume with ethyl-acetate:methanol (19:1, v/v). Working calibration solutions were prepared by evaporating the appropriate volume of the stock solution and diluting with 1.00 ml of mobile phase. Standard solutions were stored at 4 °C.

Equipment. UV Tubes, both with 15 W power and two different wavelengths (368 nm and 254 nm) providing UVA and UVC radiations, were obtained from local sources. UV Sterilizer (35 x 50 x 20 cm), fitted with UVA and UVC tube (both 15 Watt) and having slots at distances of 15 cm and 30 cm for the trays, was also obtained from local sources.

EXPOSURE TO UVA AND UVC IRRADIATION

The UV sterilizer was fitted with UVA and UVC tube (both 15 Watt) on the top. Twelve different experiments were carried out, whereby 100 g of maize sample was spread in a layer not more than 1 cm thick in two different trays and was exposed to UV radiations. The exposure of the samples was done by varying the following parameters:

1) UV lamps: UVA and UVC lamps (both 15 Watt) were used for sample irradiation
2) Contamination level of sample: samples were naturally contaminated with DON at two concentration levels (≈2mg/kg and≈5 mg/kg)
3) Distance from UV lamps: The exposure of the contaminated samples was done by placing the samples at two different distances i.e. at 15 cm and 30 cm from the tube
4) Duration of exposure: 30, 60 and 120 minutes. Samples were mixed during irradiation at exactly half of exposure time (15, 30 and 60 minutes).

Maize samples were exposed for different time duration, i.e. 30, 60 and 120 minutes at both the distances, using both, UVA and UVC lamps. Samples were exposed in duplicates at each of the time interval. Samples were analyzed in duplicate for DON content. Control samples were analyzed in triplicate.

DON DETERMINATION

Sample preparation. Control samples and irradiated samples were milled in a laboratory mill and a portion was taken for analysis. 25 g of sample was extracted with 100 ml acetonitrile:water (84:16, v/v) by mixing on a magnetic stirrer (Ultra Turrax T18, IKA, Staufen, Germany) for 30 minutes. The extract was filtered through filter paper (Filtros Anoe, Blue label, Maidstone, UK) and 3 ml was cleaned up on Mycosep^{TM} 225 (Trich) columns (Romer Labs. Inc., Union, MO, USA). The cleaned-up extract was evaporated just to dryness.

HPLC analysis. The equipment consisted of an HPLC system – Agilent Technologies 1260 series (Agilent Technologies, USA) with a DAD detector (Agilent Technologies, USA) and a column Hypersil ODS (100 x 4.6 mm i.d., particle size 5 μm, Agilent Technologies, USA).

The DON analysis was performed after evaporation. The residue was redissolved in 300 μl of mobile phase, and 15 μl aliquot of the solution was injected into the HPLC system. The mobile phase consisted of an isocratic mixture of water:acetonitrile (84:16, v/v), with a flow rate of 0.6 ml/min. The detection of DON was performed at 220 nm. The mobile phase was filtered through a 0.45 μm regenerated cellulose membrane filter (Agilent Technologies, USA). Identification of DON was done by comparing the retention times and spectra of DON from samples with those of the standards.

Validation performance and quality control. Selectivity, linearity, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were determined to test the validity of the procedures used for DON determination. Validation parameters were calculated according to a procedure regulated by the European Commission (2006). To determine the accuracy the of used method, certified reference material TR-D100 (Trilogy, USA) was used. Average recovery value was 92.3% which is within acceptable limits according to European Commission (2006). LOD for DON determination in maize samples was 0.025 mg/kg while LOQ was 0.074 mg/kg. Precision was estimated in terms of repeatability and reproductivity. Both parameters can be described as "acceptable" according to the European Commission (2006). Therefore, the method was suitable for the determination of DON in maize samples.

RESULTS AND DISCUSSION

The average DON content in control samples was 1.902 and 5.334 mg/kg. These values were used for comparison with results of irradiated samples in order to calculate DON reduction levels as treatment efficiency quantification. Results of DON content in irradiated samples are summarized in Table 1 and the results of DON reduction levels (percent of initial concentration left in sample after UV irradiation) are presented in Table 2. In general, it can be said that UV irradiation reduced DON content in the treated samples, but when observed in detail, there were some uncertainties.

Table 1. DON content (mg/kg) after UV irradiation.

DON content in control sample (mg/kg)	**1.902**				**5.334**			
UV wavelength (nm)	**254**		**368**		**254**		**368**	
Treatment distance (cm)/time (min)	**15**	**30**	**15**	**30**	**15**	**30**	**15**	**30**
30	1.132	0.666	0.984	0.308	5.634	2.114	3.246	3.263
60	1.468	0.643	0.802	0.776	4.429	7.911	4.142	5.160
120	1.599	2.330	0.983	0.805	2.989	0.891	1.771	4.993

In the treated sample with DON content of 1.902 mg/kg, the reduction level increased with increasing UV exposure time at both 254 nm and 368 nm wavelengths. This may be explained by more difficult DON reduction, lower availability, and quantification at low contamination levels. On the other hand, in the irradiated sample containing 5.334 mg/kg of DON, the reduction levels decreased with increasing UV exposure time at 254 nm, which was as expected, but the results were quite inconsistent at wavelength of 368 nm.

Table 2. DON reduction levels (%) after UV irradiation.

DON content in control sample (mg/kg)	**1.902 (100%)**				**5.334 (100%)**			
UV wavelength (nm)	**254**		**368**		**254**		**368**	
Treatment distance (cm)/time (min)	**15**	**30**	**15**	**30**	**15**	**30**	**15**	**30**
30	60	35	52	16	106	40	61	61
60	77	34	42	41	83	148	78	97
120	84	123	52	42	56	17	33	94

Regarding reduction levels, the highest reduction from 5.334 to 0.891 mg/kg (17%) was observed at 254 nm during the exposure time of 120 minutes at 30 cm from UV source. The lowest reduction level could not be determined because there were found irradiated samples with DON contents higher than the ones in control samples. The highest level found in the irradiated samples was 7.911 mg/kg (148% of initial content). The most probable reason for this is a very inconsistent DON distribution in naturally contaminated samples. Chelkowski (1998) stated that the difference in DON content among kernels, originating from the same sample, can be up to ten times, depending on whether the kernels are damaged by *Fusarium* spp. or looked healthy. This fact was most likely the cause of inconsistent results at wavelength of 368 nm, especially in the sample containing 5.334 mg/kg of DON.

CONCLUSION

The preliminary results presented in this paper showed that the UV irradiation is an applicable procedure for DON degradation in maize samples, but more accurate scientific evidence had to be provided. Further research should be directed towards UV irradiation in artificially inoculated samples with consistent DON distribution. In this manner, the results on DON reduction are going to be more reliable and accurate. Another way of possible improvement of the results reliability is by enlarging the amount of the irradiated sample and analyzing a higher number of replicates. It is also needed to increase the frequency of samples mixing during the irradiation procedure, so all sample parts can get as equally irradiated as possible.

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PRELIMINARNI REZULTATI O DEGRADACIJI DEOKSINIVALENOLA U KUKURUZU PRIMENOM UVA I UVC OZRAČIVANJA

Izvod: Cilj ovog rada je bio da se ispita mogućnost korišćenja UV zračenja za degradaciju deoksinivalenola (DON-a) u uzorcima prirodno kontaminiranog kukuruza. Studija je sprovedena na različitim udaljenostima kontaminiranog kukuruza od izvora UVA (368 nm) i UVC (254 nm) zračenja, kao i tokom različitih perioda izloženosti kontaminiranog kukuruza UV zračenju. Za sprovođenje postupaka ozračivanja, korišćena su dva kontrolna uzorka kukuruza, sa sadržajima DON-a od 1,902 mg/kg i 5,334 mg/kg. Uzorci su izloženi UVA i UVC zračenju na dva različita rastojanja od UV lampe (15 i 30 cm) tokom tri perioda izloženosti (30, 60 i 120 minuta). Nakon ozračivanja, ispitan je sadržaj DON-a kako bi se utvrdio nivo redukcije ovog mikotoksina. Generalno, rezultati su pokazali smanjenje sadržaja DON-a u tretiranim uzorcima, ali oni nisu bili u potpunosti konzistentni. Najverovatniji razlog za to je bio veoma nejednaka distribucija DON-a u prirodno kontaminiranim uzorcima. Iz tog razloga, moraju se uraditi dalja istraživanja pri čemu je potrebno izvršiti ozračivanje veštački inokulisanih uzoraka sa pravilnijom distribucijom DON-a, količina ozračenog uzorka treba da bude povećana, a potrebno je i analizirati veći

broj ponavljanja. Kao dodatno poboljšanje, potrebno je povećati i učestalost mešanja uzoraka prilikom postupka ozračivanja.

Ključne reči: *degradacija, deoksinivalenol, kukuruz, ultra-violetno ozračivanje.*

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