PAPER

CORN GRAIN BRUSHING FOR DEOXYNIVALENOL REDUCTION

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ABSTRACT

For the purpose of deoxynivalenol (DON) reduction in corn samples, a laboratory brusher was developed. The brusher had two main parts, motionless screen and fast rotating brush. Corn kernels were placed on the motionless screen of the brusher, and fast rotating round shaped polypropylene bristle brush was put into the motionless screen in order to clean the surface of the kernels. The experiment consisted of 27 brushing trials with variations in process parameters (DON concentration, brushing time, and speed of rotating brush). Brushing of corn that is DON infected resulted in the reduction of DON concentration in all samples. The maximal limit of DON reduction at optimal conditions was 83.6%. Thus, the presented process can be considered as highly efficient. The proposed technology did not cause any changes in the physical appearance of kernel, nor were damages observed on kernel surface during the brushing process. Also, there were no whole grain losses detected in any of the process parameter combination.

Keywords: brushing, corn, decontamination, deoxynivalenol

1. INTRODUCTION

Well-known and frequently mentioned secondary metabolites of fungi, mycotoxins, commonly occurred in cereal grains and other food and feedstuffs. They belong to the most toxic contaminants among a wide range of food commodities (COUNCIL FOR AGRICULTURAL SCIENCE AND TECHNOLOGY, 2003; KOUROUSEKOS and THEODOSIADOU, 2018). These highly harmful compounds are extremely thermostabile; for instance, pure aflatoxin B1 can be destroyed only at temperatures above 160°C (KARLOVSKY *et al.*, 2016). During "field to fork" chain, contaminated food materials are not exposed to temperature, which can lead to mycotoxin decontamination, or if it is exposed, the process does not last long enough to be decontaminated. Moreover, conditions on the field as well as conditions during further manipulation with cereal products oftentimes promote fungal growth and mycotoxin production.

Exposure to the mycotoxins is somehow unavoidable (MAGAN and OLSEN, 2006). Chronic consumption of contaminated goods can adversely affect human and animal health and can be lethal to some animal species. Main toxic effects of mycotoxins are teratotoxicity, carcinogenicity, hepatotoxicity, nephrotoxicity, embryotoxicity and immunosuppression (PESTKA, 2010; ANFOSSI *et al.*, 2010).

One of the best ways to manage contamination is to apply pre-harvest and post-harvest preventive strategies, that is by avoiding the emergence of mycotoxins. However, when the material is already contaminated, mycotoxins should be inactivated, destroyed, or removed from the commodity. Besides, the nutritive value and acceptability of the products should be preserved, and technological properties of the product should be retained (AVANTAGGIATO, 2012). Nowadays, various procedures are applied for mycotoxin decontamination, which can be divided into three main groups: chemical, biological, and physical.

Chemicals, such as oxidizing agents, chlorinating agents, acids, and alkali, have been used for the deactivation of mycotoxins. Despite the fact that some of these agents were found to be effective, the chemical procedures are not widely used due to their negative effect on product palatability and nutritive value, potential toxicity, high operational costs, and long operational time (AMÉZQUETA *et al.* 2009; ZAKI *et al.*, 2012).

Biological detoxification, which is based on biotransformation and/or biodegradation principles, is widely used in the deactivation of mycotoxins contaminated feedstuffs, as well as for food and beverages. Enzymes, microorganisms or a specific organic compound derived from microorganisms interact with mycotoxins in the gastrointestinal tract of animals to form a non-toxic stable complex. To enable sufficient contact surface between contaminated substrate and biological additive, grain materials are usually milled before the addition of bio-binder. Therefore, when biological detoxification is performed, the grains could not be preserved in a whole kernel form, nor the effects of the binders observed before grains are consumed by animals (KOLOSOVA and STROKA, 2011; KARLOVSKY *et al.*, 2011).

Physical treatment of contaminated materials includes, sorting, screening, milling, washing, irradiation, thermal treatments, adsorbing, etc. Some of these procedures can be found in conventional grain storage and/or processing technologies, with the main purpose of improving the nutritional quality of grains, and not to remove mycotoxins (TRENHOLM *et al.*, 1991). Taking into account that most of these operations do not destroy mycotoxins, the effect of physical procedures is often weak to moderate (KABAK, 2009; MANAFI and KHOSRAVINIA, 2012; MARIN *et al.*, 2013). Generally, the selected method for mycotoxin decontamination should be efficient, relatively simple, inexpensive, and not time-consuming.

For the purpose of mycotoxin decontamination in grain, a team of scientists at the Institute of Food Technology, University of Novi Sad, Serbia, developed an intensive laboratory grain brusher. Preliminary trials have shown a significant effect of the brushing process on the aflatoxin (AF) removal (ČOLOVIĆ *et al.*, 2013). Although there is a considerable number of papers referring to the effects of the scouring process on mycotoxin reduction, the literature on the effects of cleaning grain surface without damage of pericarp is limited (SCHAARSCHMIDT and FAUHL-HASSEK, 2018).

Considering all the aforementioned facts, the main objective of this study was to present a new method of physical detoxification, primarily. Furthermore, we wanted to investigate the influence of processing parameters (processing time (t), and rotational speed (v)) on reduction rate of DON in maize samples naturally contaminated with this mycotoxin in three different concentrations and to determine the optimal conditions of brush procedure applied. Response Surface Methodology (RSM) was used since it was proven to be an effective tool for optimizing a wide variety of processes (LIAUDANSKAS *et al.*, 2018). Experimental results were subjected to analysis of variance (*ANOVA*) to show the relationship between applied assays.

2. MATERIALS AND METHODS

2.1. Material

Samples of mycotoxin contaminated corn were collected from commercial warehouses within the Serbian Northern Province of Vojvodina. Sampling of three corn samples was performed in accordance with Commission Regulation 401/2006 (European Commission, 2006). The total amount of aggregate sample (10 kg) was homogenized using Nauta mixer, model 19387 (Nauta patenten, Netherlands). After homogenization, the aggregate sample was quartered to get 3000 g of the representative sample. Obtained representative corn samples were again homogenized using rotation drum mixer (model SYTH0,05, Muyang Group, China) and quartered to get sub-samples of 100 g per contaminated sample (27 sub samples of each contaminated sample). Initial DON concentrations in naturally contaminated samples were 7.5 mg/kg (DON1), 10.6 mg/kg (DON2), and 14.8 mg/kg (DON3).

2.2. Chemicals and reagents

Acetonitrile used for HPLC analysis (all HPLC grade, purity ≥ 99.9%) was purchased from Merck (Darmstadt, Germany). Ultra-pure water was produced by Milli-Q purification system (Milli-Q from Millipore, USA). DON (concentration of 100 μg/mL) standard was purchased from Sigma-Aldrich (Steinhem, Germany). Standard solutions were prepared in acetonitrile and stored at –10 °C. Those solutions were used for solvent based calibration and for fortification of blank corn samples.

2.3. Sample preparation

Sub-samples of 100 g were ground to a 1 mm particle size using laboratory mill (KnifetecTM 1095 mill, Foss, Hoganas, Sweden), and additionally quartered to obtain sub-samples of 25 g. The sub-samples were further extracted with 100 ml of acetonitrile:water (84:16, v/v) and shaken vigorously for 30 minutes on a laboratory Griffin flask shaker (Griffin and George, Wembley, England). The extract was filtered through no. 4 filter paper (Whatman, Maidstone, UK). Three ml of extract was cleaned up on MycoSep® 225

Trich for DON determination (Romer Labs, Inc., Union, MO). The cleaned-up extract was evaporated to dryness (Reacti-Therm ™ manifold, Thermo Fisher Scientific, Inc., USA), and reconstituted in 300 ml of mobile phase.

2.4. HPLC analysis of DON and validation procedure

Agilent 1200 (Agilent Technologies Inc., USA) HPLC instrument system equipped with diode array detector (DAD) was used for determination of DON. The detection was performed at 220 nm. Agilent column Eclipse XDB-C18, 1.8 μm, 4.6 x 50 mm was used. The mobile phase consisted of an isocratic mixture of water-acetonitrile (80:20, v/v), with a flow rate of 0.25 ml/min. Each sample was analysed in duplicate.
Before application in the experiment shown, HPLC/DAD method was developed and validated for DON determination. The validation parameters were determined and calculated according to EU Commission Decision procedure (2002/657/EC) by analysing certified reference material as well as spiked corn samples at three different levels. CRM with certified DON content of 900±100 μg/kg (D-W-164) was supplied by Trilogy Analytical Laboratory (Trilogy® Reference Material, Washington, USA). The results of methodology validation are shown in Table 1. As can be seen, the obtained validation parameters were in compliance with the recommendations given in Regulation 2006/401/EC (EC, 2006).

Table 1. Validation parameters for DON determination.

Validation parameters	CRM	Spiked concentration (μg/kg)		
		1000	5000	10000
RSD_r	6.56	7.12	8.15	5.94
RSD_R	8.81	9.22	10.1	6.56
Recovery	96.7	94.5	92.5	95.1

CRM - certified reference material (naturally contaminated corn, D-W-164, Trilogy® Reference Material, Washington, USA).
RSDr- relative standard deviation calculated under repeatability conditions (%).
RSD_R- relative standard deviation calculated under reproducibility conditions (%).

2.5. Processing

Contaminated corn, in a kernel form, was subjected to the brushing process. For this purpose, laboratory brusher developed at the Institute of Food Technology, University of Novi Sad, Serbia, was used (Fig. 1). The brusher had two main parts, fast rotating brush (A) and motionless screen (B). Corn kernels were placed onto the motionless screen to cover the surface (approximately 100 g) of the brusher (laboratory test sieve was used for this purpose), and fast rotating round shaped polypropylene bristle brush was put down into the motionless screen. The purpose of the corn kernel brushing was to remove dust from the surface of whole kernels, and to brush it out together with broken kernels through the openings in the motionless screen. Aspiration of the dust is provided from the bottom side of the screen by connecting it to the central aspiration system, in order to facilitate separation, and to prevent excessive dusting, as well as inhalation of toxic substances. Brushing time was set at 30, 60, and 90 s, respectively, while speed of rotating brush was set at 400, 800, and 1200 rpm, respectively.

Figure 1. Laboratory grain brusher. A - fast rotating brush, B - motionless screen.

Extent of DON reduction (E_{DON} (%)) was calculated using the following equation:

$$E_{DON} = \left(1 - \frac{C_{DON\ \text{Red.1–3}}}{C_{DON\ 1–3}}\right) \cdot 100\% \qquad \text{(Equation 1)}$$

2.6. Experimental design and statistical analysis

The experimental data used for the study of experimental results were obtained using a 3^2 full factorial experimental design; each of the 3 specific DON contaminated samples were processed at 2 parameters and at 3 levels. Independent experimental factors for each of the samples are shown in Table 2.

Table 2. Independent experimental factors and their levels.

Experimental factor	Symbol	Coded factor's level		
		-1 (low)	0 (centre)	+1 (high)
t – Brushing time (s)	X_1	30	60	90
v – Speed of rotating brush (rpm)	X_2	400	800	1200

Descriptive statistical analyses of all the obtained results were expressed by means, for each treatment. Collected data were subjected to ANOVA to explore the effects of process variables. The evaluation of RSM and ANOVA of the obtained results was performed using Statistica software version 12 (StatSoft Inc. 2013, USA)®.

The experimental data used for the analysis were derived according to RSM. The main advantage of RSM is a reduced number of experimental runs that provide sufficient information for statistically valid results. The RSM equations describe the effects of the test variables on the observed responses, determine test variable interrelationships and represent the combined effect of all test variables in the observed responses, enabling the experimenter to make efficient exploration of the process (ČOLOVIĆ *et al.*, 2016, BRLEK *et al.*, 2013).
The following second order polynomial (SOP) model was fitted to the experimental data. Six models of the following form were developed to relate six responses (Y) and three process variables (X), for each of the different mixtures.

$$Y_k = \beta_{k0} + \sum_{i=1}^{2} \beta_{ki} \cdot X_i + \sum_{i=1}^{2} \beta_{kii} \cdot X_i^2 + \beta_{k12} \cdot X_1 \cdot X_2, \quad \text{(Equation 2)}$$

where: β_{k0}, β_{ki}, β_{kii}, β_{k12} are constant regression coefficients; Y_k, deoxynivalenol concentration after reduction (C_{DONRed}), for three initial concentrations of deoxynivalenol ($DON_{1\text{-}3}$), while $X1$ - brushing time (t); $X2$-speed of rotating brush (v).

3. RESULTS AND DISCUSSION

The results for the effects of brushing process on DON reduction have been shown in Fig. 2 from the least to the most contaminated samples, respectively. The brushing of infected corn resulted in the reduction of concentration of DON in all samples, matter on DON concentration, brushing time or speed of rotating brush.
The best results of brushing for DON concentration of 7.5 mg/kg (DON_1) and 14.8 mg/kg (DON_3) were recorded at the highest levels of rotating speed and the longest time of brushing, respectively. The samples before and after brushing for rotating speed of 1200 rpm and brushing time of 90 s, as well as tailings collected from the aspiration system has been shown in Fig. 3. However, samples contaminated with concentration of 10.6 mg/kg (DON_2) have shown the highest level of decontamination at rotating speed of 1200 rpm and a duration of 60 s. Looking at Fig. 2, it is clear that the results of decontamination level were unpredictable for rotation speed of 400 and 800 rpm. The reason for that is probably due to the insufficient rotating speed of brush, so decontamination level is rather accidental and not dependent on brushing time. At rotation speed of 1200 rpm, decontamination level increased with the increase in processing time. A similar conclusion can be made for DON_2 concentration. Yet, decontamination level for DON_2 samples differ insignificantly for rotation speed of 1200 rpm and processing time of 60 and 90 s (approx. 81% and 78% respectively). It seems that for this DON concentration, sufficient time of brushing for maximum decontamination is 60s at a brushing speed of 1200 rpm.
An average mycotoxin reduction of 27 samples was 50.9%, showing that the grain brushing process was very effective in the removal of DON. Also, only five of 27 samples had DON reduction less than 40%, while only two of those had DON reduction less than 20%. Generally, for a short brushing time and low speed of rotating brush, DON reduction was lower. VISCONTI *et al.* (2004) showed that concentration of DON is the highest in the outer layers of grains such as bran. This also explains why intensive cleaning of the outer layers of corn applied in our study reduced DON concentration.

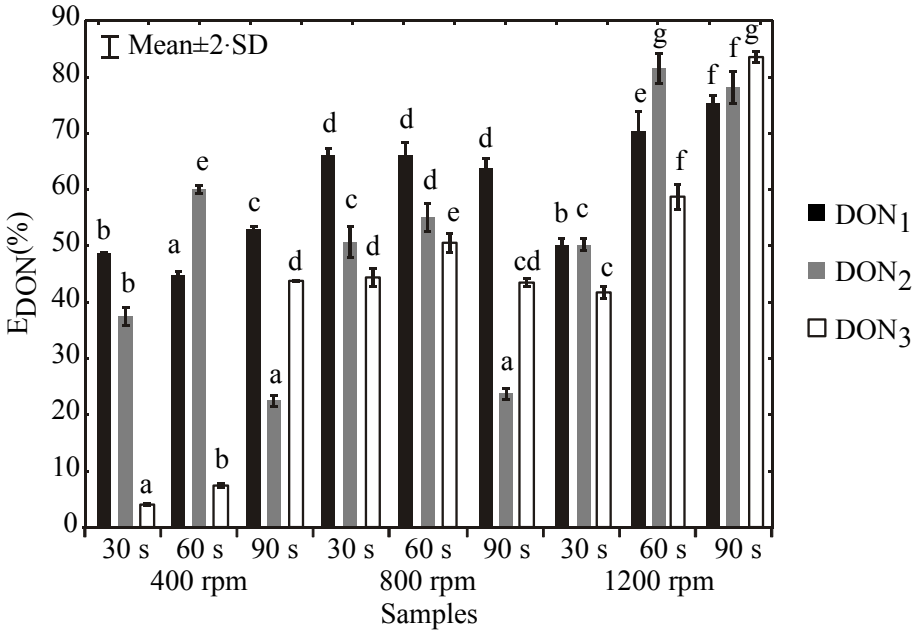

Figure 2. Extent of DON reduction (%) by application of grain brushing process.
a-g - Different letters within the same set of samples show significantly different means of observed data at $p<0.05$ level.

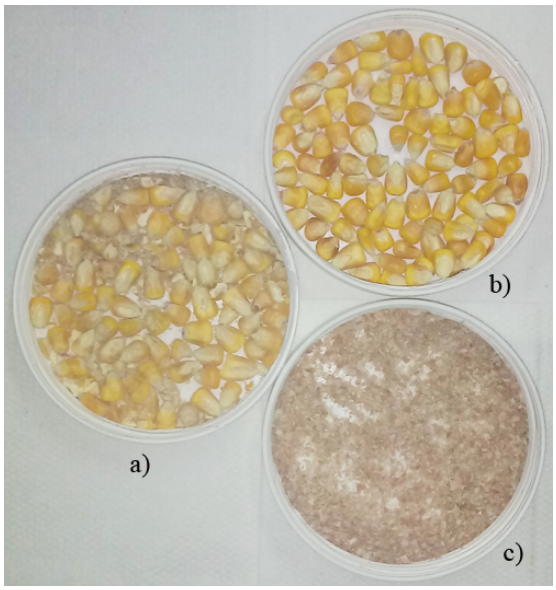

Figure 3. The infected sample, contaminated with heavy DON concentration (a), the sample after brushing treatment (for rotating speed of 1200 rpm and brushing time of 90 s) (b), and the brushed tailings collected from the aspiration system (c).

Physical dehulling of the outer layer of maize can reduce AF decontamination by 92% (SIWELA *et al*., 2005). However, this method removes natural protection of kernel and it does not preserve its integrity. For any combination of process parameters, changes in physical integrity of kernel or damages to the kernel surface were not observed during the brushing process. Since there is no damage of the grain, kernels could preserve its biological function and its natural protection from microbial contamination. Therefore, it is

reasonable to expect that kernels decontaminated by brushing have a longer shelf life than kernels decontaminated with similar mechanical treatments, which include surface breakage of kernels.

It cannot be neglected that, by this method, none of the total mass of treated kernels was removed and as a result, in that way, there were no material losses. As WU and MUNKVOLD (2008) stated in their paper that deals with the economic costs of mycotoxin's presence in feed, removing of screenings and broken kernels from maize after sieving reduced DON contamination by 73%, however, mass loses were extensively high and accounted for approx. 69%. Removal of contaminated maize can also be performed by flotation and density segregation (GRENIER *et al.*, 2014). Fungal damaged kernels are mostly, also, mycotoxin-contaminated. Since they have different physical properties than non-infected kernels, they can be separated by density segregation or by fractionation on so-called gravity tables. However, these methods are not mycotoxin specific, so kernels contaminated with fungi, but without presence of mycotoxins are also removed.

A group of Italian authors showed in their paper an electronic optical technique of sorting infected and healthy apricot kernels based on the discoloration of contaminated kernels (ZIVOLI *et al.*, 2016). Although, they succeeded in removing up to 59% of aflatoxin accumulated in naturally-contaminated samples, the obtained results were highly variated, such that the proposed method cannot be considered as reliable and need to be improved. It may be less effective in comparison with the manual method of sorting, such as removal of contaminated grains, which is, on the other hand, highly time demanding.KUSHIRO (2008) in his review paper showed that DON concentration could be decreased by 86% when infested wheat kernels are removed. On the other hand, PARK (2002) combined removal of extensive mould growth kernels with the cleaning of maize kernels for reduction of AF content, and obtained a reduction of 40% to 80%. That is similar to the maximal mycotoxin reduction in our study, where extensive mould growth kernels were not removed. However, the maize was not infected with the same mycotoxin. Same author also used dry milling process for fractionation of AF B1 content. Highest levels of mycotoxin were found in the germ and hull fractions. Grits, low-fat meal and low fat flour contained only 6 to 10% of AF. Since a prerequisite for fractionation is to comminute the kernel, authors of present study changed the physical structure of corn kernels. Also, fractionation resulted in the concentration of toxin in separate fractions, without removal of mycotoxins from the material.

Washing grains with tap water significantly reduced the mycotoxin level and this can be applied to food and feeds (FANDOHAN *et al.*, 2005). Yet, costs of drying grains are too high, so it is reasonable to use this method only prior to wet milling or ethanol fermentation. The same goes for rinsing and flotation techniques.

Most of the physical methods of decontamination can be generally considered as efficient, but the major problems occur with implementation of these processes on a commercial scale. As earlier mentioned, milling, combined with sieving causes significant mass losses, and most of other established methods are time consuming, which cannot be said for the presented method of kernel brushing. Laboratory grain brusher presented in this study has been shown to be generally efficient on a small scale level. Meanwhile, development of a continuous semi-industrial scale brusher is in progress with the accent on maintaining same efficacy as it was on a lab scale device.

ANOVA shows the significant effects of independent variables to the responses (Table 3). The SOP models for all variables were found to be statistically significant and the response surfaces were fitted to these models. The linear term of v was the most influential in the DON_1 reduction calculation (statistically significant, at $p < 0.10$ level). The prediction of DON reduction was influenced by a linear term of v and the quadratic term of t (both

statistically significant at $p < 0.10$ level). The linear term of v was very influential for DON reduction calculation ($p < 0.05$), for DON_3.
All SOP models had an insignificant lack of fit tests, which means that all the models represented the data satisfactorily (Table 3).
A high r^2 is indicative that the variation was accounted and that the data fitted satisfactorily to the proposed model (SOP in this case). The coefficients of determination for DON reduction prediction were very good and showed the good fit of the model to experimental results.

Table 3. ANOVA calculation for DON reduction during grain brushing process.

	dF	$C_{DONRed\ 1}$	$C_{DONRed\ 2}$	$C_{DONRed\ 3}$
t	1	124.519	32.491	1082.945
t^2	1	1.660	947.083**	42.844
v	1	407.825**	1350.000**	2764.210*
v^2	1	135.942	279.609	78.972
t × v	1	108.160	462.656	1.097
Error	3	161.312	442.659	757.681
r^2		0.828	0.874	0.840

Abbreviations: t - brushing time, v - speed of rotating brush, dF - degrees of freedom. + significant at $p<0.01$ level, *significant at $p<0.05$ level, **significant at $p<0.10$ level, error terms have been found to be statistically insignificant.

Using these models, the contour plots of the extent of DON reduction (E_{DON}) were plotted and superimposed to ascertain the optimum processing conditions (Fig. 4).

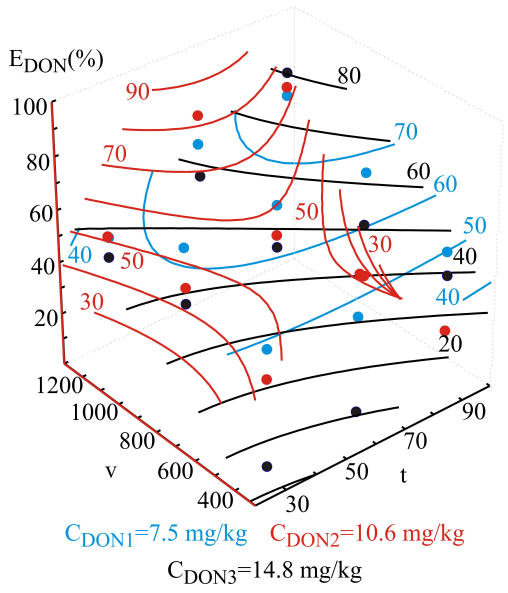

Figure 4. Optimum region obtained after superimposing the contour plots of the system response.

Optimization of the process is performed to ensure rapid processing conditions with high DON reduction. Optimum operating conditions were derived with a few iterative steps in finding processing parameters that gave the highest reduction of DON. Contour plots of the extent of DON reduction showed that maximum reduction was obtained at higher initial concentration and higher speed of rotating brush, as was expected. The optimal conditions for DON reduction were t = 90 s, v = 1200 rpm, which consistent with experimental results. The maximal extents of DON reduction at optimal conditions were: 75.3%, 78.2% and 83.6%, for the initial DON concentration of 7.5 mg/kg, 10.6 mg/kg and 14.8 mg/kg, respectively.

4. CONCLUSIONS

This study presented fast technological process which successfully detoxified corn without causing any changes in the physical integrity of the kernel, nor damages of kernel surface. Therefore, the presented laboratory brusher could easily grow into its industrial version, especially due to its simple construction. The higher reduction of DON was obtained for the higher initial concentration, longer polishing time, and higher speed of rotating brush. Further, brushing of corn infected with DON resulted in reduction of concentration in all processed samples. It is important to note that the proposed process did not lead to whole grain loss, but only fine particles, unlike other effective physical decontamination methods, such as sieving or fractionation.

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REFERENCES

Amézqueta S., González-Peñas E., Murillo-Arbizu M., and López de Cerain A. 2009. Ochratoxin A decontamination: A review. Food Control 20(4):326.

Anfossi L., Baggiani C., Giovannoli C., and Giraudi G. 2010. Mycotoxins in food and feed: extraction, analysis and emerging technologies for rapid and on-field detection. Recent Pat. Food Nutr. Agric. 2: 40.

Avantaggiato G. 2012. Mycotoxin decontamination in the food/feed chain, in detection and decontamination techniques for mycotoxins in the food/feed chain. Training Course, Bari, Italy.

Brlek T., Pezo L., Voća N., Krička T., Vukmirović Đ., Čolović R., and Bodroža-Solarov M. 2013. Chemometric approach for assessing the quality of olive cake pellets. Fuel Process. Tech. 116: 50.

EC, 2002. Commission Decision (2002/657/EC) of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Off. J. Eur. Comm. L221:8-36.

EC, 2006. Commission Regulation N. 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Off. J. Eur. Comm. L 70:12-34.

Council for Agricultural Science and Technology. 2003. Mycotoxins: risks in plant, animal and human systems. Task Force Report, Ames, USA 39:1-217.

Čolović D., Čolović R., Lević J., Ikonic B., Vukmirović D., and Lević Lj. 2016. Linseed-sunflower meal co-extrudate as a functional additive for animal feed – extrusion optimization. J. Agric. Sci. Technol. 18:1761.

Čolović R., Bursić V., Vuković G., Vukmirović Đ., Lazić S., Lević J., and Bagi F. 2013. Reducing the level of mycotoxin in corn by removal of fines. Proc. Nat. Sci., Matica Srpska 124:59.

Fandohan P., Zoumenou D., Hounhouigan D., Marasas W., Wingfield M., and Hell K. 2005. Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin. Int. J. Food. Microbiol. 98(3): 249.

Grenier B., Loureiro-Bracarense A.-P., Leslie J., and Oswald I. 2014. Physical and chemical methods for mycotoxin decontamination in corn. In: "Mycotoxin reduction in grain chains". J. Leslie and A. Logrieco (Ed.), p. 116. John Wiley and Sons, NY, USA.

Kabak B. 2009. The fate of mycotoxins during thermal food processing. J. Sci. Food Agric. 89(4):549.

Karlovsky P., Suman M., Berthiller F., De Meester J., Eisenbrand G., Perrin I.,Oswald I., Speijers G., Chiodini A., Recker T., and Dussort P. 2016. Impact of food processing and detoxification treatments on mycotoxin contamination. Mycotoxin Res. 32:179.

Kolosova A. and Stroka J. 2011. Substances for reduction of the contamination of feed by mycotoxins: a review. World Mycotoxin J. 4(3):225.

Kushiro M. 2008. Effects of milling and cooking processes on the deoxynivalenol content in wheat. Int. J. Mol. Sci. 9(11):2127.

Kourousekos G. and Theodosiadou E. 2018. Effects of Aflatoxins on Male Reproductive System: A Review. J. Hell. Vet. Med. Soc. 66(4):201.

Liaudanskas M., Zymonė K., Viškelis J., and Janulis V. 2018 Optimisation of the extraction of flavonoids from apples using response surface methodology. Ital. J. Food Sci. 30 (1):89-101.

Magan M. and Olsen N. 2006. Mycotoxins in food: detection and control. CRC Press-Woodhead Publishing, NY, USA.

Manafi M. and Khosravinia H. 2012. Effects of aflatoxin on the performance of broiler breeders and its alleviation through herbal mycotoxin binder. J. Agr. Sci. Tech. - Iran 15.1:55.

Marin S., Ramos A., Cano-Sancho G., Sanchis V. 2013. Mycotoxins: Occurrence, toxicology, and exposure assessment. Food Chem Toxicol 60:218.

Park D. 2002. Effect of processing on aflatoxin. Adv. Exp. Med. Biol. 504:173.

Pestka J. 2010. Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Arch. Toxicol. 84:663.

Schaarschmidt S. and Fauhl-Hassek C. 2018. The Fate of Mycotoxins During the Processing of Wheat for Human Consumption. Compr. Rev. Food Sci. F. 17:556.

Siwela A., Siwela M., Matindi G., Dube S., and Nsiramazanga N. 2005. Decontamination of maize by dehulling. J. Sci. Food Agric. 85:2535.

Trenholm H., Charmley L., Prelusky D., and Warner R.. 1991. Two physical methods for the decontamination of four cereals contaminated with deoxynivalenol and zearalenone. J. Agric. Food Chem. 39:356.

Visconti A., Haidukowski E., Pascale M., and Silvestri M. 2004. Reduction of deoxynivalenol during durum wheat processing and spaghetti cooking. Toxicol. Lett., 153.1:181.

Wu F. and Munkvold G. 2008. Mycotoxins in ethanol co-products: modeling economic impacts on the livestock industry and management strategies. J. Agric. Food Chem. 56 (11): 900.

Zaki M., El-Midany S., Shaheen H., and Rizzi L. 2012. Mycotoxins in animals: Occurrence, effects, prevention and management. J. Toxicol. Environ. Health Sci. 4:13.

Zivoli R., Gambacorta L., Piemontese L., and Solfrizzo M. 2016. Reduction of Aflatoxins in Apricot Kernels by Electronic and Manual Color Sorting. Toxins 8(1):26.

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