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The effect of thermal processing on the reduction of deoxynivalenol and zearalenone cereal content

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ABSTRACT

Fusarium mycotoxins deoxynivalenol (DON) and zearalenone (ZEN) often contaminate cereals and cereal by-products. Certain thermal processing methods used in the food industry show promising results in terms of reduction of cereal contamination with mycotoxins. In order to establish the degree of DON and ZEN reduction in naturally contaminated cereals (maize, wheat and oat), this study investigated the effects of cooking, roasting and extrusion cooking, performed at different temperatures (100 – 220 °C) and for a different length of time (10 – 30 min) on these mycotoxins concentrations. Before and after the treatment, cereal samples were analysed for DON and ZEN concentrations using enzyme-linked immunosorbent assay (ELISA). In comparison to cooking, which achieved only a negligible mean mycotoxin reduction (of up to 8% for DON and 11% for ZEN), roasting and extrusion cooking resulted in a significantly more pronounced mycotoxin reduction (of up to 40% for DON & 46% for ZEN and of up to 75% for DON & 80% for ZEN, respectively) ($p < 0.05$). The results show similar effects of thermal processing on all of the studied cereals ($p > 0.05$), suggesting that extrusion cooking can be considered as an effective thermal method capable of reducing mycotoxin content in cereals.

Introduction

Mycotoxins of the *Fusarium* species are very often associated with cereal contamination that may primarily occur in the field during the period of cereals' growth and harvesting. The level of contamination varies across geographical areas and climatic regions, and is influenced by the formation of moulds, moisture level, temperature, aeration, the presence of insects, and mechanical damage of stored cereals (Placinta et al., 1999; Pleadin et al., 2015). Mycotoxin contamination becomes an issue primarily during rainy years characterised by substantial temperature changes (Pleadin et al., 2012). Because of health-related risks and possible financial shortcomings (IARC, 1993; Kabak et al., 2006; Pleadin et al., 2015), the maximal permitted levels (MPLs) of these mycotoxins in food are stipulated under the pertaining legislation (Commission Regulation 1881/2006). Earlier studies have revealed that, because of climate, cereals are at constant risk of *Fusarium* mycotoxin contamination both, in Croatia and neighboring countries of this European part (Domijan et al., 2005; Pleadin et al., 2013; Pleadin et al., 2017).

Among *Fusarium* mycotoxins deoxynivalenol (DON) is the most widespread mycotoxin primarily produced by *F. graminearum* and *F. culmorum* in cooler climates (Sudakin, 2003; Eriksen and Pettersson, 2004). The absorption of DON causes damage to the epithelial cells of intestinal tract and kidney damage as

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well (Richard, 2007). Zearalenone (ZEN) as uterotropic and estrogenic compound also represents secondary metabolite of *Fusarium* moulds, produced mostly by *F. graminearum*, *F. culmorum*, *F. crookwellense*, *F. equiseti*, and *F. semitectum*. This mycotoxin is often encountered at very high concentrations, especially in maize (Bottalico, 1998). As the consumption of DON and ZEN contaminated products may cause the poisoning known as mycotoxicosis and induce teratogenic, carcinogenic, neurotoxic, estrogenic or immune-suppressive effects (Kabak et al., 2006), contamination of food, feed and their ingredients can significantly affect human and animal health (IARC, 1993; Bottalico, 1998).

A stable nature of mycotoxins often leads to the contamination of products in downstream processes, including raw cereals and finished products (Ryu et al., 2008).Therefore, physical, chemical and biological methods have been used for their decontamination (Jackson and Bullerman, 1999). Among other treatments, those processes which are implemented by the food industry, such as sorting, trimming, cleaning, cooking, baking, frying, roasting, flaking and extrusion, have usually decreased the level of mycotoxin contamination, but mostly failed to eliminate the toxins completely (Schwake-Anduschus et al., 2010; Scudamore et al., 2007; De Angelis et al., 2013).

In industrial food processing, the combination of processing length and temperature has been shown to be crucial for the reduction of mycotoxin content in the finished product (Boudra et al., 1995; Jackson et al., 1996a; Jackson et al., 1996b; Ryu et al., 2003). The reduction of mycotoxins is generally correlated with the amount of heat employed in the process; however, heat energy alone may fail to completely eliminate mycotoxins during food processing (Ryu et al., 2008). While conventional food preparation that takes place at temperatures of up to 100 °C has a negligible effect on most mycotoxins, higher temperatures used when frying, roasting, toasting and extrusion have been shown to be capable of decreasing mycotoxin contamination (Kaushik, 2015; Karlovsky et al., 2016). Among thermal processing techniques listed above, roasting and extrusion show promising results in terms of lowering mycotoxin concentrations, although very high temperatures are needed to achieve substantial reductions of mycotoxin concentrations (Singhal et al., 2010).

Data on the efficiency of mycotoxin reduction achieved by virtue of thermal processing techniques are inconsistent and have been obtained under various conditions. In order to investigate the degree of thermal reduction of *Fusarium* mycotoxins DON and ZEN in naturally contaminated cereals, this study used cooking, roasting and extrusion, each running at different temperatures and for a different length of time.

Materials and Methods

Sampling and sample preparation

In the first step of this study analyses of cereals were performed in order to find the samples contaminated with DON and ZEN in the concentrations "as highly as possible". The highest contaminated samples were further used for treatment using three thermal processing methods. For this purpose, during 2017 a total of 50 maize (n = 20), wheat (n = 20) and oat (n = 10) samples were randomly sampled from the fields in the Northern and Eastern Croatia and analysed for DON and ZEN concentrations. All of the sampled cereals had been grown in the crop season 2016 and had not undergone any physical or thermal treatment other than drying, cleaning and sorting prior to sampling. Sampling and sample preparation of unprocessed cereals were performed in full line with the Commission Regulation 401/2006, laying down the methods of sampling to be exercised within the frame of the official control of mycotoxin levels in foodstuffs.

The samples were stored in a cool and dry place and transported to the laboratory within 48 h. The prepared test portions (500 g per sample) were ground into a fine powder having a particle size of 1.0 mm by using an analytical mill (Cylotec 1093, Tecator, Sweden), and then stored at 4 °C pending analyses. In all cereal samples, the moisture content was determined before and after thermal processing according to the ISO standard 712:2009, by taking a 5-g sample and heating it in an oven at 105 ± 2 °C.

Chemicals, standards and reference materials

Determination of mycotoxin concentrations was performed using competitive RIDASCREEN® ELISA kits: DON (Art No R5906) and Zearalenone (Art No R1401). Each kit contains a micro-titre plate with 96 wells coated with antibodies, standard solutions containing different concentrations of mycotoxins, an enzyme conjugate, an anti-antibody, a substrate and a chromogen solution (urea peroxide/tetramethylbenzidine), a stop solution, and washing and dilution buffers.

DON (Art No D 0156) and ZEN (Art No Z2125) standards were purchased from Sigma Aldrich Chemie GmbH (Steinheim, Germany). The certified reference material (CRM) of maize was manufactured by Fapas (T04209QC, York, England) with the mean values and

ranges of DON (mean value: 1,779 μg/kg; acceptable range 1,257 to 2,301 μg/kg) and ZEN (mean value 344 μg/kg; acceptable range 214 to 473 μg/kg) assigned. All chemicals used for sample preparation were of an analytical grade.

Determination of mycotoxins

DON and ZEN concentrations in cereals before and after the treatment were determined using competitive ELISA test kits as instructed by the kit manufacturer (R-Biopharm, Darmstadt, Germany). The ELISA tests were performed using a ChemWell auto-analyser (Awareness Technology Inc. 2910, Palm City, USA), the absorbance thereby being measured at 450 nm. In order to determine mycotoxin concentrations in the sampled material, standard curves were plotted for each mycotoxin analysed. When establishing the final mycotoxin concentrations in each sample, the pre-established dilution factor and the mean recovery rate were taken into account (Pleadin et al., 2017). The quality control was performed with each analysis of each mycotoxin using the CRM analysed in two replicates, so as to check whether the obtained concentrations fall within the ranges assigned by the manufacturer.
Maize, wheat and oat samples found to be the most contaminated with DON and ZEN underwent thermal processing in terms of cooking, roasting and extrusion. Selected samples with the highest mycotoxins concentrations were firstly thermally processed in three parallel runs and then analysed for mycotoxins in three replicates.

Thermal processing

Cooking and roasting: During the cooking process, the above-mentioned samples (100 g) were cooked for 10, 20 and 30 minutes, each in its own glass cup filled with 500 mL of boiling water (96 °C). After that, the cereals were filtered and the samples were left to dry overnight. As for roasting, the samples (100 g) were roasted in an oven (LV9/11/P 320, Nabertherm, Lilienthal, Germany) at three different temperatures (180 °C, 200 °C and 220 °C for 30 min). Once cooked and roasted, the cereals were milled into a fine powder having a particle size of 1.0 mm using an analytical mill (Cyclotec 1093, Tecator, Hoganas, Sweden), and subsequently analysed for mycotoxin concentrations.
Extrusion cooking: Before the extrusion cooking, all samples were milled in an IKA MF10 laboratory mill having a 2-mm sieve. Blend preparation was performed based on 1 kg d. m. The samples were conditioned to 25% moisture by spraying with an estimated amount of distilled water and continuous mixing in a laboratory mixer (Kenwood KMM020, JVCKenwood, Uithoorn, the Netherlands). The prepared mixtures were then put into plastic bags (one bag per sample) and stored overnight in a refrigerator at 4 °C in order to equilibrate the moisture. Before the extrusion, the samples were exposed to room temperature. The prepared samples were extruded using a laboratory single-screw extruder (Brabender GmbH, Model 19/20DN, Duisburg, Germany) at three different temperature profiles: 135/150/150 °C; 135/170/170 °C and 135/190/190 °C (dosing/compression/ejection zone of the extruder). Other extrusion parameters of relevance, which had remained unchanged during the processing, were as follows: screw: 4:1; die: 4 mm; screw speed: 100 rpm; dosing speed: 40 rpm. After the extrusion, the samples were air-dried overnight.

Data analysis

Statistical data analysis was performed using the Statistica Ver. 10.0 Software (StatSoft Inc. 1984-2011, Tulsa, OK, USA) and made use of the analysis of variance (ANOVA), the statistical significance thereby being set at 95% ($p = 0.05$).

Results and Discussion

Ranges of DON and ZEN concentrations determined in randomly sampled cereals investigated in order to find the most contaminated cereals which were further subjected to reduction, are shown in Table 1. Maximal obtained concentrations of mycotoxins in contaminated cereals were as follows: the maize sample - 875 μg/kg of DON and 182 μg/kg of ZEN, the wheat sample - 713 μg/kg of DON and 64 μg/kg of ZEN and the oat sample - 428 μg/kg of DON and 59 μg/kg of ZEN. In order to establish the degree of reduction of *Fusarium* mycotoxins DON and ZEN, on the highest naturally contaminated cereal samples, this study made use of thermal processing methods including cooking, roasting, and extrusion, carried out at different temperatures for a different length of time.
In general, cooking involves high temperatures and boiling in water, which both could cause destruction and dissipation of mycotoxins (Kaushik, 2015). Several studies have reported about the effect of cooking on the reduction of *Fusarium* mycotoxins in contaminated cereals (Kamimura et al., 1979; Kushiro, 2008). The degree of DON and ZEN reduction achieved in this study during various cooking times (10 – 30 min) at the temperature of 96 °C is presented in Table 2.

Table 1. Mycotoxin concentrations determined in cereal samples

Cereals	Range (min – max) of concentration (µg/kg)	
	DON	ZEN
Maize (n =20)	25 - 875	10 - 182
Wheat (n= 20)	32 - 713	15 - 64
Oat (n = 10)	45 - 428	6 - 59

DON – deoxynivalenol; ZEN - zearalenone

Table 2. Mycotoxin reduction achieved by cooking of contaminated cereals

Cereals [a]	Mycotoxins after cooking	Cooking time (96 °C) [b]		
		10 min	20 min	30 min
Maize	DON (µg/kg)	873	880	846
	DON R (%)	NR	NR	3
	ZEN (µg/kg)	177	180	169
	ZEN R (%)	3	1	7
Wheat	DON (µg/kg)	715	712	654
	DON R (%)	NR	NR	8
	ZEN (µg/kg)	65	61	58
	ZEN R (%)	NR	5	9
Oat	DON (µg/kg)	430	414	370
	DON R (%)	NR	3	14
	ZEN (µg/kg)	60	58	50
	ZEN R (%)	NR	2	15
Mean reduction	**DON (%)**	**0**	**1**	**8**
	ZEN (%)	**1**	**3**	**11**

[a] Concentrations of mycotoxins in cereals before treatment: 875 µg/kg of DON and 182 µg/kg of ZEN in maize; 713 µg/kg of DON and 64 µg/kg of ZEN in wheat; 428 µg/kg of DON and 59 µg/kg of ZEN in oat

[b] Deoxynivalenol (DON) and zearalenone (ZEN) concentrations are presented as the mean value of three replicates; R - reduction; NR – not reduced

The results show that after 10-min cooking the DON content was not reduced at all; after 20 minutes, the reduction of up to 3% was achieved, while after 30-min cooking, the DON content reduction of 3-14% was achieved. In comparison with DON, an equal or a slightly more pronounced reduction was observed for ZEN, with the ZEN content reduction of up to 3% after 10-min cooking, 1-5% after 20-min cooking, and 7-15% after 30-min cooking. With a longer cooking time (30 min), the reduction of DON content increased to 8%, and that of the ZEN content to 11%. A significantly higher ($p < 0.05$) degree of reduction of both mycotoxins was obtained during prolonged (30-min) cooking, while a significant difference in the degree of reduction of the studied mycotoxins in the studied cereals was not observed ($p > 0.05$). The results indicate a high thermal stability of both DON and ZEN at the temperature used with cooking (96 °C), regardless of the applied cooking time (10 - 30 min).

Kushiro (2008) concluded that DON is highly water-soluble, so that its levels can be drastically reduced by dissolution, provided that the contaminated food is cooked in a larger quantity of boiling water that later gets to be discarded (which is the case, for example, with spaghetti cooking). In the study quoted above, the reduction of DON content in spaghetti ranged from 20% to 33%, and was up to 37% in semolina and up to 77% in cleaned wheat. The authors concluded that the reduction of mycotoxins is dependent on the cooking time, temperature, pH, recipe, food additives and some other factors. The results of the Kushiro study, which achieved a low degree of DON and ZEN reduction, can be explained by the cooking conditions, primarily by the fact that cereals were processed as whole grains and were cooked in a small amount of water (500 mL). The mycotoxin content reduction obtained within the frame of the above mentionedstudy is comparable to the (low) degree of mycotoxin reduction in some earlier investigations (Scudamore, 2008).

Roasting is a processing method that uses dry heat generated by an open flame, oven, or other heat source, which can enhance flavour through caramelisation and

Maillard browning taking place on the product surface (Kaushik, 2015). The degrees of DON and ZEN reduction obtained by 30-min roasting at three different temperatures (180 – 220 °C) are presented in Table 3.

Table 3. Mycotoxin reduction achieved by roasting of contaminated cereals at different temperatures

Cereals [a]	**Mycotoxins after roasting**	**Temperature of roasting (30 min)** [b]		
		180 °C	**200 °C**	**220 °C**
Maize	DON (µg/kg)	815	716	558
	DON R (%)	7	18	36
	ZEN (µg/kg)	156	147	122
	ZEN R (%)	14	19	33
Wheat	DON (µg/kg)	625	558	403
	DON R (%)	12	22	43
	ZEN (µg/kg)	45	38	29
	ZEN R (%)	30	41	55
Oat	DON (µg/kg)	388	301	255
	DON R (%)	9	30	40
	ZEN (µg/kg)	47	43	30
	ZEN R (%)	20	27	49
Mean reduction	**DON (%)**	**9**	**23**	**40**
	ZEN (%)	**21**	**29**	**46**

[a] Concentrations of mycotoxins in cereals before treatment: 875 µg/kg of DON and 182 µg/kg of ZEN in maize; 713 µg/kg of DON and 64 µg/kg of ZEN in wheat; 428 µg/kg of DON and 59 µg/kg of ZEN in oat
[b]Deoxynivalenol (DON) and zearalenone (ZEN) concentrations are presented as the mean value of three replicates; R - reduction

Table 4. Mycotoxin reduction achieved by extrusion cooking of contaminated cereals at different temperatures

Cereals [a]	**Mycotoxins after extrusion**	**Regime of extrusion** [b]		
		135-150-150 °C	**135-170-170 °C**	**135-190-190 °C**
Maize	DON (µg/kg)	398	351	295
	DON R (%)	55	60	66
	ZEN (µg/kg)	68	63	51
	ZEN R (%)	63	65	72
Wheat	DON (µg/kg)	346	275	206
	DON R (%)	51	61	71
	ZEN (µg/kg)	33	30	21
	ZEN R (%)	48	53	67
Oat	DON (µg/kg)	115	87	55
	DON R (%)	73	80	87
	ZEN (µg/kg)	25	18	ND
	ZEN R (%)	58	69	100
Mean reduction	**DON (%)**	**60**	**67**	**75**
	ZEN (%)	**56**	**62**	**80**

[a] Concentrations of mycotoxins in cereals before treatment: 875 µg/kg of DON and 182 µg/kg of ZEN in maize; 713 µg/kg of DON and 64 µg/kg of ZEN in wheat; 428 µg/kg of DON and 59 µg/kg of ZEN in oat
Deoxynivalenol (DON) and zearalenone (ZEN) concentrations are presented as the mean value of three replicates; R - reduction; ND – not detected

The results achieved by roasting show the reduction of DON to range from the minimal 7-12% attained at the roasting temperature of 180 °C, over 18-30% attained at the roasting temperature of 200 °C, to the maximal 36-40% obtained at the roasting temperature of 220 °C. The attained degrees of ZEN reduction were as follows: 14-30% at 180 °C, 19-41% at 200 °C and 33-55% at 220 °C. With the increase in the roasting temperature, DON reduction increased from the average value of 9% to 40%, while that of ZEN rose from 21% to 46%, indicating a higher thermal stability of DON as compared to ZEN. In comparison to cooking, this study resulted in a significantly higher DON and ZEN reduction when roasting was applied, which can be explained by earlier observations that processing time and temperature are crucial for the reduction of mycotoxin content in a final product (Ryu et al., 2008). As roasting included higher temperatures (180 – 220 °C *vs* 96 °C used with cooking) and longer processing times (30 min *versus* 10, 20 and 30 min of cooking), a superior effect of roasting over cooking is understandable.

Earlier investigations have shown that roasting in an electrical oven for 6-10 min reduces mycotoxins for up to 40%. Should the roasting be prolonged to 15 minutes, a higher degree of mycotoxin reduction of up to 75% can be attained (Bokhari and Aly, 2009). However, investigations into the possibility of DON and ZEN reduction using roasting are very scarce and are actually mostly concerned with baking. Traditional baking of cookies and bread yielded DON reduction ranging from 0 to 35% (Scott et al., 1984; Young et al., 1984). Gilbert et al. (1984) showed that 80% of DON survived in both spiked and naturally contaminated wheat, whereas the study of Abbas et al. (1985) resulted in variable DON losses spanning from 19 to 69%. Tanaka et al. (1986) concluded that baking at 170 °C could not degrade ZEN. It was concluded that DON is very stable during thermal processing such as baking and that it is only partially degraded to DON-related chemicals whose toxicological effects have not been thoroughly studied yet (Kushiro, 2008). Unlike other food processing methods, extrusion cooking simultaneously employs a high amount of heat, high pressure and mechanical shear energy, and has been extensively used in the production of breakfast cereal snacks and animal feedstuffs (Ryu et al., 2008). Cereals that pass through an extruder under pressure undergo mechanical shearing stresses at an elevated temperature, and rapidly expand when forced through the outlet die (Guy, 2001). The whole process usually takes less than few minutes and represents one of the fastest-growing food-processing operations in the recent years, due to its many advantages. Apart from its main goal in terms of improving product quality, extrusion may also significantly improve product safety, because of its potential to reduce mycotoxin levels in cereals (Castells et al., 2005). The application of this method decreases the mycotoxin content in dependence of the moisture content, screw centrifugation, extruder geometry, die temperature, die size, screw speed and additives (Castells et al., 2005), while the extrusion temperature was found to be a minor factor of influence. During extrusion, mycotoxins are subjected to both high temperatures and chemical reactions mediated by free radical mechanisms, so that they might be susceptible to some degree of breakdown that yields variable effects. In general, high moisture content and high shear rates degrade mycotoxins more efficiently if the processed sample is poor in ingredients or additives (Scudamore et al., 2008a; Scudamore et al., 2008b).

The reduction of DON and ZEN achieved in this study by extrusion cooking of contaminated cereals under three different extrusion regimens (135-150-150 °C, 135-170-170 °C 135-190-190 °C) is shown in Table 4. Following the extrusion cooking under three different temperature regimens but with the same moisture content (25%), the accomplished reduction of DON ranged from the minimal 51-73% under the 135-150-150 °C regimen, over 60-80% under the 135-170-170 °C regimen, to the maximal 66-87% under the 135-190-190 °C extrusion regimen. The extrusion resulted in ZEN reduction ranging from 48-63% under the 135-150-150 °C regimen, over 53-69% under the 135-170-170 °C regimen, to 67-100% under the 135-190-190 °C extrusion regimen. With the increase in the extrusion temperature, the reduction of DON increased from the average of 60% to 75% and that of ZEN from 56% to 80%, indicating a complete reduction of ZEN at the highest applied temperature. A significant difference in the effects of extrusion on the reduction of the studied mycotoxins in the studied cereals was not noted, except for the reduction of DON in oat, which was significantly higher in comparison with that obtained in maize and wheat.

Ryu et al. (1999) investigated ZEN reduction achieved by extrusion and determined that reduction to be 66-83% if mixing was applied and 65-77% if no mixing was applied. Within that study frame, the grits were extruded at the temperatures of 120, 140 and 160 °C. The results of our study are comparable to those of the study referenced above. In the investigation by Castells et al. (2005), a 83 %-reduction of ZEN achieved by extrusion cooking of maize was observed, while in case of DON the maximal reduction did not exceed 55%. However, some studies show that DON and ZEN were mostly stable during extrusion of wholemeal wheat flour over the temperature range of 140 - 180 °C, with moisture content of 15 – 21%

(Scudamore et al., 2007). Also, no reduction of DON content was observed in wheat flour following a simple extrusion (Scudamore et al., 2008). It was pointed out that the inconsistency of the results presented in the literature may be a consequence of failure to control or report all conditions under which the extrusion process was taking place. For example, chemical breakdown taking place during an extrusion process is related to the duration of the process, so that the loss of a mycotoxin will depend on the residence time of the material in the extruder.

Conclusions

The study results show that, among the applied thermal processing methods (cooking, roasting and extrusion cooking), the highest reduction of *Fusarium* mycotoxins DON and ZEN was attained by extrusion cooking. While cooking does not significantly reduce DON and ZEN, roasting appears to reduce their concentrations more efficiently, whereas extrusion cooking seems to be superior over both, since it resulted in high to an almost complete mycotoxin reduction in all cereals. The reduction of mycotoxins was found to be dependent on the applied temperature regime and the duration of the processing time and did not significantly differ between the processed cereals. Future studies are needed in order to optimise all factors that influence mycotoxin degradation through extrusion cooking as the most efficient thermal processing method, so as to improve its potential in reduction of mycotoxin content in cereals. Also, some other food industry methods, such as backing, should be performed in order to examine the influence of thermal methods on the reduction of these mycotoxins more thoroughly as often contaminants of cereals and cereal based products.

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