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Article

Fate of Deoxynivalenol in different corn cultivars; From Grains to Bread

Abstract: The objectives of the current research were to determine the levels of deoxynivalenol (DON) in four different cultivars of corn and subsequently to investigate the fate of DON during processing steps involved for the production of cornbread. The samples (n = 30) of each cultivar which were found positive were selected for the study. The average level of DON was ranged from LOD to 650 µg/kg. The amount of DON in cornflour samples were ranged from LOD to 630 µg/kg and insignificantly lower than the levels found in corn grain samples ($p \geq 0.05$). Furthermore, the levels of DON in corn dough samples were insignificantly higher than the levels in cornflour samples ($p \geq 0.05$), with levels ranged from LOD to 645 µg/kg. However, the amount of DON in cornbread samples was significantly different from the levels found in corn grains samples ($p \leq 0.05$), with levels ranged from LOD to 611.5 µg/kg. The percentage reduction of DON in grains to cornbread samples was 22.4%, 35.6%, 44.5%, and 42.6% in type 1, type 2, type 3, and type 4 cultivars, respectively. The highest dietary exposure and hazard quotient (HQ) of DON was 0.13 and 0.17 µg/kg bw/d, in male and female individuals resulted from the consumption of cornbread samples, respectively.

Keywords: deoxynivalenol; corn; cultivar; grains; flour; bread; Pakistan

Key Contribution: The DON levels in different corn grain cultivars ranged from LOD to 650 µg/kg (average level 150.4 ± 18.5 µg/kg). The levels of DON in cornflour were significantly lower than the levels in corn grains ($p \geq 0.05$). The dough levels were insignificantly slightly higher than the levels in the cornflour sample ($p \geq 0.05$). The percentage reduction of DON from grains to cornbread was 22.4%, 35.6%, 44.5%, and 42.6% in type 1, type 2, type 2, and type 4 cultivars of corn.

1. Introduction

Food containing natural or synthetic toxic substances like putrescine, histamine, tyramine, and mycotoxins are creating serious concerns for human health as well as for animals and effects the international and national trade of food commodities [1-2]. In tropical and subtropical countries, the main food safety concern is mycotoxins' presence in food or feed, which is a byproduct of various types of fungi [3-4]. It is estimated that about 25–50% of the crops harvested worldwide are infected with mycotoxins. In the current situation, mycotoxins are considered the most critical chronic dietary risk factor as compared to pesticide residues or food additives [5].

The trichothecenes family's an important class and closely linked to vomitoxin is deoxynivalenol (DON) mycotoxin, and it is mainly produced by *Fusarium* species, especially *F. graminearum* and *F. culmorum*, [6-10]. The DON is also known to produce similar forms of other toxins, recognized as masked mycotoxins, which usually remain undetected during the analytical process [11]. In the light of experimental research on animals and humans, International Agency for Research on Cancer (IARC) has classified DON as a group 3, human carcinogenic compound [12]. The toxic effects may include harmful health complications such as neurotoxic, teratogenic, immunosuppressive, and embryotoxic [13]. The European Commission (EC) has established a permissible limit of 1250 µg/kg of DON in unprocessed cereal, 1750 µg/kg in unprocessed corn, oats, and wheat, 750 µg/kg in flour and pasta, 500 µg/kg in cereal snacks, cookies, breakfast cereals, pastries, 500 µg/kg in bread, and 200 µg/kg in infant food [14].

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The food processing steps including, cleaning, trimming, sorting, milling, brewing, cooking, frying, baking, roasting, alkaline cooking, flaking, and nixtamalization (corn tortilla processing), remarkably affect the levels of mycotoxins in food [15]. With a production of 5.8 million tons, corn is the third most staple food used for human consumption after wheat and rice in Pakistan [4]. The corn used for human consumption is in the form of cornbread. Recently, with the investigation of masked mycotoxins in food, especially cereal products, there is increasing interest to estimate the level of DON on each step during the processing of bakery products [16].

In our previous studies, the presence of aflatoxins (AFs) and ochratoxin A (OTA) in rice [17], mycotoxins in cereal and derived products [18]; AFs in different rice cultivars [19], AFs, OTA, and zearalenone (ZEN) in breakfast cereal [20], AFs and ZEN in wheat-derived products [21] and AFs and OTA in rice, corn, and products [22] have indicated that the considerable amount of these toxins present in cereals products. However, no study was conducted to study the fate or distribution of DON during processing steps from grains to bread in bakery products. The fact is that the mycotoxins are very stable in high temperatures and therefore present in end products [24]. Therefore, it is a continuation of our previous study [4] to estimate DON levels during each processing step of bread making and assess the exposure of DON using cornbread. The results would reflect the actual exposure assessment of DON and help to establish regulations.

2. Results and Discussion

2.1. Method validation parameters

The recoveries analysis of DON in corn flour, corn dough, and cornbread samples was performed by fortifying 70, 150, 200, and 500 μg/kg. The recoveries were calculated as described in Eq-1.

$$\text{Percentage recoveries} = \frac{Con\ in\ fortified\ sample - Con\ in\ without\ fortified\ sample}{Known\ amount\ (\mu\frac{g}{kg})} \times 100 \qquad (1)$$

The percentage recoveries of DON in various matrixes were above 80% in all fortifying samples, and relative standard deviation ranged from 14 to 25% (Table 1). The Detection limit (LOD) and quantification limit (LOQ) were 30 and 60 μg/kg, respectively. The coefficient of determination (R^2) was 0.9910. The repeatability and reproducibility were 13 to 24% and 19 to 25%, respectively.

The recoveries of DON in the current research agreed with a previous study [4], which documented recoveries of DON in corn and products ranged from 81.5% to 91%, and a relative standard deviation of 11 to 28%. However, the LOD and LOQ were 25 and 50 μg/kg, respectively. In another study [10], good recoveries of DON were observed in wheat and corn products with levels ranged from 80.8 to 92.6%, RSD ranged from 9 to 27%. However, the LOD and LOQ values were slightly higher, i.e., 50 and 100 μg/kg, respectively, as compared to the present study. Zhang and Wang, [11] from China, have observed excellent recoveries of DON in wheat flour (99.4%), wheat dough (92.9 %), noodles (91.9%), cooked noodles (103.2%), baked bread (90.2%), and in water 107.4%. They have documented very low LOD and LOQ, i.e., 0.5 and 1.5 μg/kg for solid matrix and 0.2 and 0.5 μg/kg for water, respectively. The evolution of repeatability and reproducibility values are linked with the performance of the validated method. However, the procedure followed for reducing the sample size and the amount of sample size during analysis could affect mycotoxins' levels or create uncertainties in the developed method [23]. Therefore, the representative sample must be ensured during analysis for mycotoxins.

2.2. Distribution of DON in different corn cultivars, and during processing steps of cornbread

The results on the occurrence of DON in different corn cultivars and during each step, i.e., from grains, flour, dough, and cornbread, are shown in Figure 1. The levels of DON in different corn grain cultivars were ranged from LOD to 650 μg/kg (average level 150.4 ± 18.5 μg/kg), and the highest mean level was 164.6 ± 21.5 μg/kg in Type 1 cultivars of corn. The levels of DON in cornflour were significantly lower than the levels in corn

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grains ($p \geq 0.05$). The DON levels ranged from LOD to 630 µg/kg (average value 97.7 ± 9.5 µg/kg).

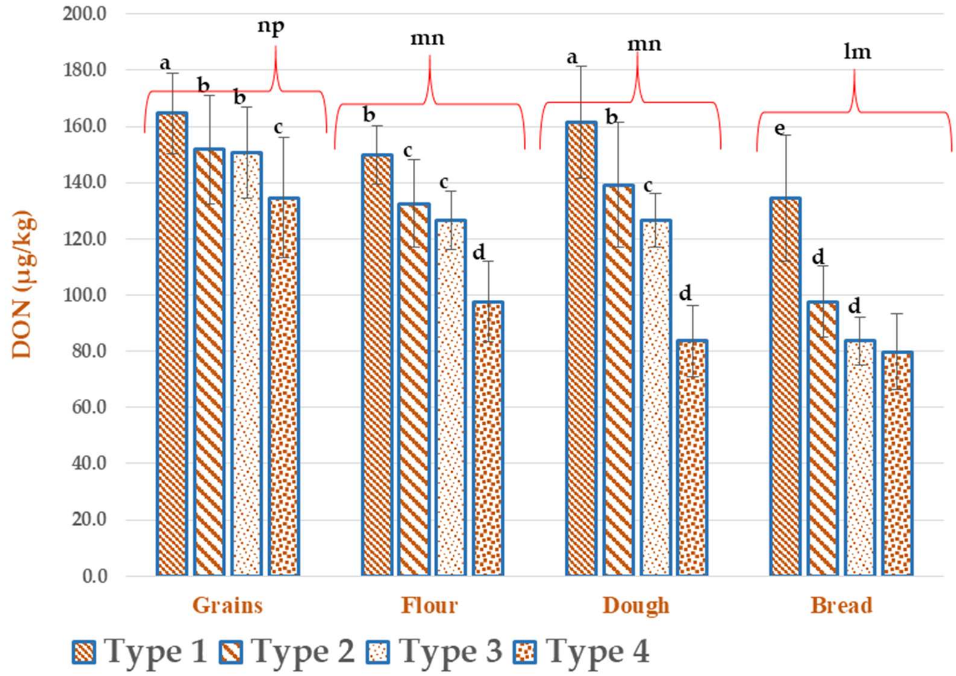

Figure 1. The distribution of DON (µg/kg) in grains, flour, dough and during preparation of corn bread. Different English letters represents a significant difference of DON levels ($p \leq 0.05$).

The concentrations of DON in corn dough were insignificantly slightly higher than the cornflour sample ($p \geq 0.05$). The dough levels in corn dough ranged from LOD to 645.1 (average level 127.7 ± 12.1 µg/kg). However, the amounts of DON in cornbread samples were significantly lower than the amount in corn grain samples ($p < 0.05$). The levels of DON in cornbread samples were ranged from LOD to 611.5 with an average value of 98.9 ±10.5 µg/kg. The percentage reduction of DON from grains to cornbread was 22.4%, 35.6%, 44.5%, and 42.6% in type 1, type 2, type 2, and type 4 cultivars of corn.

A high level ranged from 82 to 2975 µg/kg was documented in wheat samples with an average mean of 770.7 µg/kg [25]. From China, DON was detected in 100% wheat flour samples with levels ranged from 51.6 to 1308.9 µg/kg, and in Chinese steamed bread, the levels were ranged from 54.5–845.4 µg/kg [26]. Similarly, an exceedingly high level of 50 to 9480 µg/kg was documented in wheat [27]. Liu et al. [28] have observed levels of DON in wheat ranged from 2.4 to 1130 µg/kg. Considerably, very high levels ranged from 14.52 to 41157.13 µg/kg [29].

The finding of the current research was quite similar to results documented by Rahimi et al. [2]; they observed that the incidence of DON in different processing steps involved during wheat flour to baked bread and found the levels ranged 85.0 to 540.2 µg/kg in flour samples. They found that the levels of DON in the fermented and mixed dough were similar but insignificantly higher than flour ($P > 0.05$), and it was significantly lower in baked bread than in flour ($P < 0.05$). The levels of DON reduced during backing were 42.5, 54.9, 48.7, 57.1, and 49.3, for a different type of Iranian bread, i.e., Sangak, Lavash, Sokhari, Taftoon, and Barbari, respectively. Xu et al. [30] have observed 95.7% of corn germs were found to be contaminated by DON with an average amount of 449.0 µg/kg. However, the average level of DON in corn processed products including solvent extracted oil (163.7 µg/kg), cold-press oil (113.1 µg/kg), meal (1111.5 µg/kg), and cake 1175.2 µg/kg, respectively.

In the current research, the levels of DON have insignificantly ($p \geq 0.05$) increased in dough compared to initial levels in flour and grains. The previous reports have documented that additives during the preparation of dough like salt, sugar, or yeast could influence the levels of DON in dough [31]. The duration of fermentation time and temperature during dough preparation also plays a vital role in mycotoxins levels [11]. The presence of yeast in bakery products might facilitate the enzymatic conversion of DON precursors [32]. However, there are some studies, which reported a reduction of 40% in dough fermentation. Samar et al. [33] have observed the reduction of DON around 56% to 41% in Viennese bread and 41% in French bread. The emphasis is on investigating the levels of DON during the processing of bakery products, especially the origin, storage conditions of grains and flour, packaging and humidity levels, and different temperatures and sample pre-treatments.

The DON documented that the DON's stability is stable at a temperature of 120 °C, moderately and partially stable at 180 °C and 120 °C, respectively, as mentioned by The World Health Organization [34]. In the current study, the levels of DON in baked bread were significantly ($p < 0.05$) lower than the levels present in cornflour samples. The reason might be the dilution effect of other ingredients, especially water, which reduced the level of DON in the final baked bread. The reduction of 46% and 41% of DON were found in Vienna and French bread [35]. Other reports have also indicated that the levels of DON were decreased in final backed bread and observed a positive relationship between baking time and DON reduction in bakery products [36]. A reduction of 42.3% to 33% in bread (French) and 58.3 to 58.5% in Viennese type bread [37]. However, several studies have specified no change or slight change, or even increased final-backed bread levels. The reason might be explained by the fact that heating temperature might release the bonded DON into final backed bread [38].

2.3. Assessment of exposure of DON in cornbread

The assessment of DON exposure in cornbread samples from four different corn cultivars is shown in Table 2. The DON's highest exposure was 0.13 and 0.17 μg/kg bw/d, in male and female individuals from cornbread samples originated from cultivars 1. The HQ levels were also the same.

The current findings of exposure assessment in cornbread are higher than our previous study Iqbal et al. [4], which documented the highest mean value of DON was 9.68 μg/kg bw/d, and the lowest exposure was 0.92 μg/kg bw/d in cornflour samples. In another study [10], the levels of DON exposure in wheat, corn, and their products from the winter season have higher levels than the samples from summer. The exposure of DON in winter season samples was 4.78 μg/kg bw/d in cornflour, 2.14 μg/kg bw/d in cornbread, 3.56 μg/kg bw/d in boiled corn, and 1.66 μg/kg bw/d in cornflake samples, much higher levels compared to present findings. The data show that the average levels of DON in Pakistani cornbread are lower than the European Commission's maximum levels, i.e., 1 μg/kg bw/d. However, the exposure studies are mainly depending on sex, age, eating habits, environmental conditions, and seasonal variation, and all these factors may affect the levels of exposure assessments. Therefore, a comprehensive study must be conducted to include gender, eating habits, and environmental consequences during food exposure studies.

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Table 1. Method validation parameters in corn flour, corn dough and cornbread samples.

Don level	**Corn flour**				**Corn dough**				**Cornbread**			
	Recovery	**RSD**	**Precision (%)**		**Recovery**	**RSD**	**Precision (%)**		**Recovery**	**RSD**	**Precision (%)**	
µg/Kg	**(%)**	**%)**	Repeatability [a]	Reproducibility [b]	**(%)**	**%)**	Repeatability [a]	Reproducibility [b]	**(%)**	**(%)**	Repeatability [a]	Reproducibility
70	80.5	18	13	22	91.5	19	20	19	81.5	16	14	22
150	87.5	14	17	21	106.5	24	21	19	84.5	19	18	25
200	86.5	20	18	25	102.4	19	18	24	87.5	21	15	22
500	88.0	15	21	22	98.9	15	24	19	88.5	20	22	24

n = 7 samples; LOD 30 µg/kg; LOQ= 60 µg/kg; a = Repeatability; b = Reproducibility.

Table 2. Exposure assessment of DON in corn bread from different types of corn samples.

		Type 1			**Type 2**			**Type 3**			**Type 4**		
	Consumption (kg)	**DON levels**	**Exposure**		**DON levels**	**Exposure**		**DON levels**	**Exposure**		**DON levels**	**Exposure**	**IQ**
		Mean µg/kg	Mean µg/kg bw/d	IQ	Mean µg/kg	Mean µg/kg bw/d	IQ	Mean µg/kg	Mean µg/kg bw/d	IQ	Mean µg/kg	Mean µg/kg bw/d	
Male	0.07	134.5	0.13	0.13	97.7	0.09	0.13	83.6	0.08	0.13	79.7	0.07	0.13
Female			0.17	0.17		0.12	0.17		0.11	0.17		0.10	0.17

HQ = mean level of DON; Male average weight= 75 ±10 kg; Female average weight = 55 ± 8 kg.

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3. Conclusions

The results of the current finding have shown a comparatively high levels of DON in corn grains, and subsequently, in final baked bread. The levels of DON in different corn grain cultivars were ranged from LOD to 650 µg/kg (average level 150.4 ± 18.5 µg/kg). Furthermore, the levels of DON in cornflour samples were significantly lower than the levels in corn grains ($p \geq 0.05$). The levels of DON in dough samples were insignificantly higher than the levels in cornflour sample ($p \geq 0.05$). However, the levels of DON's in cornbread samples were ranged from LOD to 611.5, with an average value of 98.9 ±10.5 µg/kg. The percentage reduction of DON in grains to cornbread samples was 22.4%, 35.6%, 44.5%, and 42.6% in type 1, type 2, type 3, and type 4 cultivars of corn, respectively. The DON's highest dietary exposure was 0.13 and 0.17 µg/kg bw/d, in male and female individuals. The findings of the current research emphasized the need to conduct a more comprehensive study on DON's and its masked types produced during processing of bakery products. The information will be beneficial for people working in the food industry, traders and exporters and local consumers.

4. Materials and methods

4.1. Sampling

Almost 30 samples of each four cultivars (n =120) of corn (positive sample with DON) were selected from our previous study [4]. The samples were collected randomly from the central cities of Punjab, Pakistan, from June 2019 to December 2019. The size of each grain sample was not less than 5 kg each. The milling of corn samples was done from a commercial milling machine to relate milling process processing as close to the actual process. Then the samples were stored in plastic bags at a temperature of 25 °C. The process of preparation of dough and bread is explained in Figure 2. The grains, flour, dough and bread samples are shown in supplementary Fig S1. The ready bread was collected in sealed plastic bags and stored in a freezer at temperature (-4 °C) for further analysis.

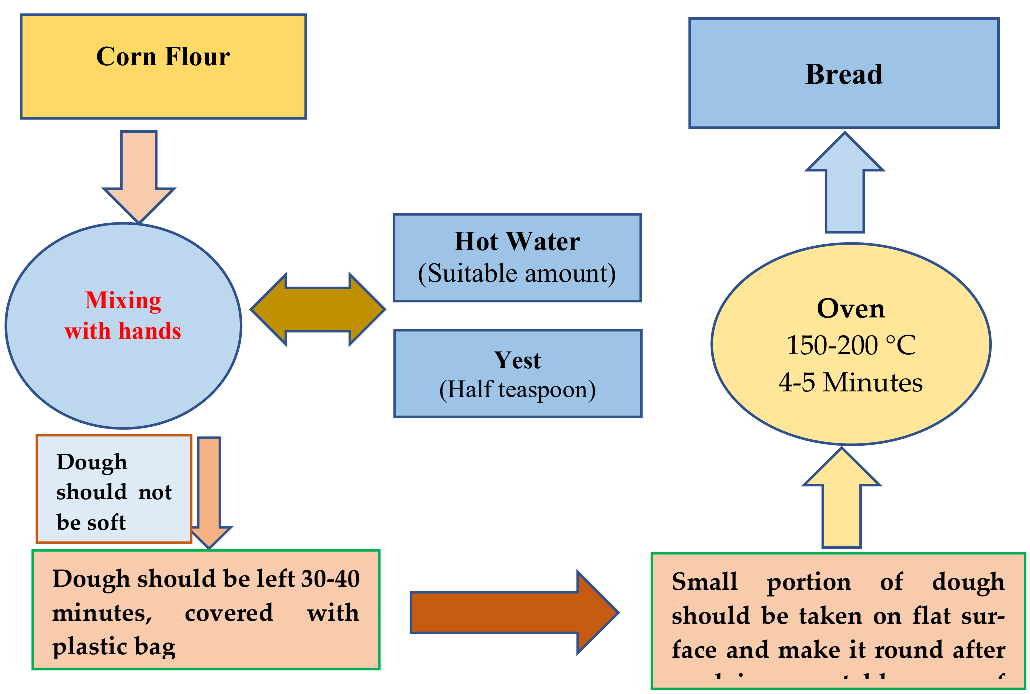

Figure 2. The process of preparation of dough and corn bread. The oven temperature is maintained constant to observe the equal effect on each sample of bread.

4.2. Chemicals and reagents

 doi:10.20944/preprints202103.0695.v1

The liquid solution of DON (100 μg/mL in acetonitrile) of 2 mL Sigma Aldrich (St Louis, MO, USA) was already present in the Food Safety lab. The solution for constructing a standard curve, prepared from 30 to 700 μg/L, was prepared from a stock solution in pure acetonitrile and stored at a temperature of -4 °C. The samples were cleaned using an immunoaffinity column (DON-Test) of VICAM (MA, USA). Furthermore, all other chemicals and reagents were HPLC grades.

4.3. Extraction and HPLC conditions

The samples were extracted of DON from corn, and products were carried from our previous method [4] with slight modifications [39]. The sample, 25g, was ground (lab blender) and homogenized in a solution of acetonitrile: water (84:16, v/v). After homogenization (3 min), the sample was filtered with Whatman (no 5) filter paper. The 2 mL portion of the filtrate was transferred in immunoaffinity columns, which were adjusted with a flow rate of 1 mL/min. The column was washed with ultrapure water (1 mL). Finally, the DON was eluted using 1 mL of pure methanol, and then the solvent was evaporated with a nitrogen stream at 25 °C. After evaporation, the residue was mixed with a mixture of 200 mL of acetonitrile: water; 90:10, v/v). The 20 μL was injected for HPLC-UV analysis. The assessment tests were analyzed using isocratic mode. The mobile phase (acetonitrile; water; 10:90 v/v) was prepared each day. The mobile phase's flow rate was 1.5 mL/min, and the DON was assessed using a UV detector (218 nm).

4.4. Exposure assessment

The exposure of DON for the Pakistani population via cornbread utilization was performed based on the risk assessment, and average DON levels were analyzed according to the following equation Eq 2 [4, 40].

$$\mathrm{ADD}_{\mathrm{DON}} = \frac{C\ DON\ in\ breadx\ IR}{bw} \tag{2}$$

$\mathrm{AD}_{\mathrm{DON}}$ = average daily dose of DON; C_{DON}= Concentration of DON in cornbread; IR= intake rate of corn bread; bw= body weight.

Based on the Pakistan Agricultural Statistics, the yearly intake of bread per person was estimated to be 0.07 kg in 2018. The hazard quotient (HQ) was based on acceptable ingestion 1 μg/kg body weight daily for DON [39].

4.5. Statistical analysis

The analysis was performed using SPSS 16.0 for Windows (SPSS, Chicago, IL, USA) software package. The normality of data was analyzed using the Shapiro-Wilk test. ANOVA and LSD tests were utilized for evaluating differences among different processing steps during the preparation of cornbread. The levels for significance difference were chosen at $P < 0.05$.

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