

ORIGINAL ARTICLE

Stability of *Fusarium* toxins during traditional Turkish maize bread production

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fumonisins; maize bread; processing; trichothecenes; zearalenone.

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Abstract

Introduction The occurrence of major *Fusarium* toxins in maize samples collected in the Black Sea region of Turkey and fate of these toxins during traditional Turkish maize bread production was investigated. **Materials and methods** Twenty maize samples were analysed for deoxynivalenol, zearalenone, fumonisins, T-2 and HT-2 toxins. Contamination profiles of maize samples were determined in order to choose the toxins to be monitored during bread production. Breads were produced from low or uncontaminated maize samples and from the mixture of highly contaminated ones. After baking, crust and crumb of the breads were separated and analysed by high-performance liquid chromatography standard methods with ultraviolet or fluorescence detection. **Results and conclusions** In total, toxin levels in 30% of the maize samples were found to be higher than the limits established by the European Union. After bread processing no significant reduction of deoxynivalenol, zearalenone and fumonisins was measured in the bread crust and crumb. According to the mass balance of mycotoxins measured in maize flour and relevant maize bread only 2.1%, 0.1% and 3.1% of the total initial amounts of deoxynivalenol, zearalenone and fumonisins, respectively, were lost. Breads were not analysed for T-2 and HT-2 as their levels in maize were quite low ($< 50 \mu\text{g kg}^{-1}$).

Introduction

Trichothecenes, zearalenone (ZEA) and fumonisins (FBs) are the major *Fusarium* mycotoxins occurring worldwide in cereal grains. Attention is increasingly given to these mycotoxins because of their toxicity and high frequency of occurrence (D'Mello *et al.*, 1999; Richard, 2007). Regulatory limits have been set within the European Union for FBs in maize and for deoxynivalenol (DON) and ZEA in cereals including wheat, maize and oats [European Commission (EC), 2007].

Fusarium toxins are generally quite stable during the commercial food processing. Because they cannot be completely destroyed, they may lead to contamination of finished cereal-based foods (Visconti *et al.*, 2004; Bullerman & Bianchini, 2007; Scudamore, 2008). Therefore, it is important to follow the levels of mycotoxins during cereal proces-

ing and to adopt preventive measures at all stages of the production chain. More information is needed on the fate of mycotoxins during production to estimate their dietary intakes (Lancova *et al.*, 2008). The stability of mycotoxins during various food processing practices, in particular thermal processing, have extensively been studied and documented throughout the world (Ryu *et al.*, 1999; Cazzaniga *et al.*, 2001; Bullerman & Bianchini, 2007; Lancova *et al.*, 2008). De Girolamo *et al.* (2001) investigated the stability of fumonisin B₁ and B₂ during corn flake processing and showed some effective decontamination. The authors reported about 60–70% reduction in FBs content during the entire process. Castelo *et al.* (1998) determined a significant decrease in FBs in canned corn and baked corn bread. However, no significant loss of FBs was observed for artificially and naturally contaminated corn-muffin mixes.

Research on the occurrence and the fate of mycotoxins during typical bakery processing technologies, specific to every single country, is of increasing interest world-wide (Neira *et al.*, 1997; Omurtag, 2001; Samar *et al.*, 2001; Juan *et al.*, 2007, 2008; Lino *et al.*, 2007). However, information on the fate of *Fusarium* toxins during traditional Turkish maize bread production is lacking.

In this research, the effect of processing on the major *Fusarium* toxins (trichothecenes, ZEA, FBs) during traditional Turkish maize bread production, typical of the Black Sea region of Turkey, was investigated. For this purpose, maize samples were collected from different provinces of the region. These samples were used to determine the occurrence of the major *Fusarium* toxins and to assess the fate of these mycotoxins during maize bread processing.

Materials and methods

Reagents and materials

All reagents were of analytical grade and solvents of high-performance liquid chromatography (HPLC) grade. Acetonitrile, methanol and toluene (for organic residue analysis) were purchased from Mallinckrodt Baker (Milan, Italy). DON, ZEA, T-2 toxin (T-2), HT-2 toxin (HT-2), 4-dimethylaminopyridine (DMAP), phosphate-buffered saline (PBS), sodium chloride, *o*-phthaldialdehyde (OPA), 2-mercaptoethanol and polyethylene glycol (PEG) (molecular weight ca. 8000) were purchased from Sigma-Aldrich (Milan, Italy). FBs mix standard solution (50.6 $\mu\text{g ml}^{-1}$ FB₁ and 49.8 $\mu\text{g ml}^{-1}$ FB₂ in acetonitrile/water, 1:1) was purchased from Biopure (Tulln, Austria) and 1-anthroylnitrile (1-AN) from Wako (Neuss, Germany). Immunoaffinity columns (DON test, T-2/HT-2; Zearala test) were obtained from Vicam (Watertown, MA, USA) and Bond-Elut strong anion exchange columns were from Varian (Harbor City, CA, USA). Ultrapure water was produced by a Millipore Milli-Q system (Millipore, Bedford, MA, USA). Filter papers (Whatman no. 4) and glass microfiber filters (Whatman GF/A) were obtained from Whatman (Maidstone, UK).

Maize samples

Maize samples (18 maize kernel and two maize flour samples, 1 kg each) were collected during January–March 2009 from market places or farms in different provinces of Black Sea region of Turkey, namely Trabzon (samples 1, 5, 6, 7, 8, 9, 10, 11, 12, 13, 17, 18), Sinop (sample 2), Rize (sample 3), Artvin (sample 4), Giresun (samples 14, 20), Samsun (samples 15, 16) and Ordu (sample 19). All the samples were stored at 4 °C until the analysis and/or bread preparation.

Before analysis, maize kernels were finely ground using a model MLI 204 Bühler laboratory mill (Bühler S.p.A, Milan, Italy) to pass a 1 mm sieve.

Preparation of standard and reagent solutions

Mycotoxin stock solutions (T-2, HT-2, DON and ZEA) were prepared by dissolving the solid commercial toxins individually in acetonitrile. For FBs the commercial solution was used. Standard solutions for calibration and spiking purposes were prepared by appropriate dilution of stock solutions, either in acetonitrile or mixtures of water–acetonitrile. OPA reagent, for pre-column derivatization of FBs, was prepared by dissolving 40 mg OPA in 1 ml methanol and diluting with 5 ml 0.1 mol l⁻¹ disodium tetraborate, followed by adding 50 μl 2-mercaptoethanol and mixing. DMAP and 1-AN solutions, for pre-column derivatization of T-2 and HT-2, were prepared in toluene at concentrations of 0.325 and 0.3 $\mu\text{g ml}^{-1}$, respectively. PBS at pH 7.4 was prepared by dissolving commercial phosphate-buffered saline tablets in distilled water.

DON analysis

DON analysis was carried out according to the method reported by MacDonald *et al.* (2005b), with slight modifications. In particular, 20 g of ground sample was extracted with 80 ml water after adding 4 g of PEG. One millilitre of the filtered extract was purified with immunoaffinity column and 1.5 ml methanol was used to elute the toxin. After evaporating the eluate to dryness at 50 °C under stream of air, the residue was reconstituted with 250 μl of the HPLC mobile phase.

For the analysis of purified extracts, HPLC (HP1100, Agilent, Palo Alto, CA, USA) with diode array ultraviolet detector set at 220 nm was used. Chromatographic separation was carried out on a reversed phase column with polar endcapping, Synergi Hydro RP, 150 \times 3 mm 4 μm particle size, Phenomenex, (Torrance, CA, USA). The mobile phase consisted of a mixture of water–acetonitrile (92:8, v/v) eluted at a flow rate of 0.5 ml min⁻¹. Injection volume was 50 μl (equivalent to 0.05 g of sample).

ZEA analysis

ZEA analysis was performed according to the procedure of MacDonald *et al.* (2005a) with slight modifications. In particular, 20 g of ground sample was extracted with 80 ml of acetonitrile–water (75:25, v/v), by blending at high speed for 2 min. After diluting with PBS (1.2:10, v/v), a 50 ml aliquot was purified with immunoaffinity column. After evaporating the purified extract under stream of air at ca.

50 °C, the residue was re-dissolved in 1 ml water–acetonitrile (6:4, v/v).

ZEA was quantified by using the same HPLC used for DON analysis, but with fluorescence detector set at $\lambda_{\text{ex}} = 274$ nm and $\lambda_{\text{em}} = 440$ nm. The analytical column was a Luna[®] pentafluorophenyl propyl (PFP) (2) column 150 × 4.60 mm, 3 µm particle size, 100 Å (Phenomenex, Torrance). The mobile phase was composed of water–acetonitrile (90:10, v/v) (A) and 0.5% acetic acid in acetonitrile (B). An isocratic composition of A:B (55:45, v/v) was eluted for 12 min followed by a linear gradient to A:B (15:85, v/v) in 3 min. From 15 to 18 min the composition was A:B (15:85, v/v) and then changed back to A:B (55:45, v/v) in 2 min and lasted 8 min. Flow rate was 0.8 ml min⁻¹. A 100 µl aliquot (equivalent to 0.15 g of sample) was injected into the chromatographic apparatus.

FBs analysis

Association of Official Analytical Chemists Official Method no. 995.15 was used for the analysis of FBs with slight modifications (Sydenham *et al.*, 1996). In particular, 20 g of ground sample was extracted with 100 ml acetonitrile–methanol–water (25:25:50, v/v/v) and the filtered extract was purified with a strong anion exchange column after adjusting the pH to 5.8–6.5 with 0.1 M KOH. The purified extract was dried and reconstituted in 1000 µl acetonitrile–water (30:70, v/v). Automated pre-column derivatization of the purified extracts with OPA reagent was performed. Detection of FBs derivatives was performed by using a ProStar HPLC system (Varian Inc., Palo Alto, CA, USA) equipped with a fluorescence detector set at $\lambda_{\text{ex}} = 335$ nm and $\lambda_{\text{em}} = 440$ nm. Liquid chromatography was performed on a reversed phase Symmetry Shield C18 column (150 mm × 4.6 mm, 5 µm particle size) (Waters, Bellefonte, PA, USA). The mobile phase was composed of acetonitrile–water–acetic acid at the ratios of 30:69:1 (A) and 60:39:1 (v/v/v) (B) (Bullerman *et al.*, 2008). A linear gradient from A:B (60:40, v/v) to A:B (12:88, v/v) was performed in 21 min. After eluting A:B (12:88, v/v) for 3 min the column was washed with 100% B for 8 min and then equilibrated with A:B (60:40, v/v) for 7 min. The flow rate was 1.0 ml min⁻¹ and a 50 µl volume of the derivatized solution (equivalent to 0.05 g of sample) was injected into the chromatographic apparatus, exactly 3 min after the addition of OPA reagent.

T-2 and HT-2 toxin analysis

T-2 and HT-2 toxins were determined by using the method of Visconti *et al.* (2005) with slight modification. After extraction, 10 ml of filtrate (equivalent to 5 g of sample)

was diluted with 40 ml of 4% (w/v) NaCl solution. After cleaning up by T-2 immunoaffinity column, the purified extract was dried and derivatized with DMAP and 1-AN solutions. The reaction mixture was dried under air stream at 50 °C and reconstituted with 1000 µl mobile phase. The same chromatographic apparatus used for FBs analysis was utilized. Excitation and emission wavelengths of the fluorescence detector were at 381 and 470 nm, respectively. Liquid chromatography was performed on a Phenyl-Hexyl Luna[®] column (150 mm × 4.6 mm, 5 µm particle size) (Phenomenex, Torrance) operating under gradient conditions with acetonitrile (A) and water (B). Linear gradient was applied as follows: the initial composition, A:B (70:30), was kept constant for 5 min, then the acetonitrile content was increased to 85% in 10 min, and kept constant for 7 min. Finally, the amount of acetonitrile was increased to 100% in 2 min and kept constant for 5 min to clean the column. The flow rate of the mobile phase was set at 1.0 ml min⁻¹. Fifty microlitre of the solution (equivalent to 0.05 g of sample) was injected into the apparatus.

Bread making

According to the results of the analysis of 20 different maize samples, two maize flour samples (blank and highly contaminated) were used for bread production. Blank flour was prepared by mixing appropriate amounts of samples 13 and 15 which resulted to contain low mycotoxin levels (i.e. FB₁ 97 µg kg⁻¹; FB₂ 28 µg kg⁻¹; ZEA, DON, T-2 and HT-2 < LOD). This flour and the resulting bread were used for the recovery experiments. Highly contaminated maize flour was prepared by mixing samples 1, 8 and 10. A subsample of this flour was dried at 55 °C for 24 h and analysed in triplicate for DON, ZEA and FBs. Mycotoxin levels were then measured on a dry weight basis. The remaining flour was used to prepare contaminated bread.

Maize bread formulation and production was performed at laboratory scale, according to the traditional Turkish homemade maize bread-making procedure. According to this formulation 900 g of maize flour was mixed with 600 ml of water (at 45 °C) and 8 g of sodium chloride without addition of any yeast. All the ingredients were accurately weighed for the mass balance calculations of mycotoxin during bread production. The mixture was then placed into a greased tray (21 cm diameter), left at room temperature for about 2 h, then baked at 210 °C. The temperature at the centre of the crumb was monitored by a thermometer. Baking was terminated after approximately 60 min, when

the temperature in the centre reached nearly 90–92 °C. Thickness of the cooked bread was 4 cm, while that of crust was 5 mm. After baking, crust and crumb parts of the breads were separated, dried at 50 °C for 24 h and ground by a laboratory mill (MLI 204 Bühler S.p.A) before analysis.

Recovery experiments

Recovery experiments were performed by using blank samples (uncontaminated or low contaminated maize flour, bread crust and bread crumb) that were spiked with a known amount of toxin before extraction. After spiking, samples were left at room temperature for 1 h to evaporate the solvent. Then the lumps formed around the spiking drops were broken by using a glass rod and extraction was performed as reported above for each mycotoxin. Recovery experiments were performed in triplicate at different spiking levels for each of the toxins. The DON spiking level was 1.5 µg g⁻¹ for maize flour and 0.5 µg g⁻¹ for both bread crust and crumb parts. These levels were 2.0 and 0.5 µg g⁻¹ for FB₁; 0.5 and 0.2 µg g⁻¹ for ZEA.

Statistical analysis

Statistical analyses were performed using SPSS 15.0 for Windows version (SPSS, Chicago, IL, USA), and significance was declared at $P < 0.05$. Independent samples *t* test was used to compare the means of the total mycotoxin levels measured in flour, crust and crumb samples.

Results and discussion

Maize samples were randomly collected from market places and farms in the northern part of Turkey, as shown

in Figure 1. In the samples, the occurrence of the principal *Fusarium* toxins (DON, ZEA, T-2 and HT-2 toxins, FB₁ and FB₂) was determined. The toxins were identified from their chromatograms by comparison with authentic standards. Quantification of the toxins was performed by comparing the peak areas to that of the calibration curves of standards. Regression coefficients (R^2) for the tested mycotoxins were in the range 0.9930–0.9996 for six-point calibration curves.

Limit of detection (LOD) values, defined as signal/noise ratio of 3, were 7.6 µg kg⁻¹ for DON, 0.4 µg kg⁻¹ for ZEA, 3.9 µg kg⁻¹ for FB₁, 3.3 µg kg⁻¹ for FB₂ and 1.0 µg kg⁻¹ for T-2 and HT-2. The limit of quantification (LOQ) values, defined as signal/noise ratio of 10, were 25.3 µg kg⁻¹ for DON, 1.3 µg kg⁻¹ for ZEA, 12.9 µg kg⁻¹ for FB₁, 10.9 µg kg⁻¹ for FB₂ and 3.3 µg kg⁻¹ for T-2 and HT-2. Recovery and repeatability results for DON, ZEA, FB₁ and FB₂ in the tested matrices (maize flour, bread crust and bread crumb) are reported in Table 1. Recovery studies were not performed for T-2 and HT-2 because the fate of these toxins was not determined during maize bread production.

In general, recovery and relative standard deviation values obtained from recovery experiments were acceptable for the tested mycotoxins and matrices, considering the requirements of analytical methods for mycotoxins stipulated by EC Regulation 401/2006 (European Commission, 2006). Low FB₁ recoveries were obtained for the crust, whereas for crumb FB₁ recovery was acceptable but FB₂ recovery was low. These recovery results were considered for the mass balance calculation of each mycotoxin during bread production.



Figure 1 Provinces of Black Sea region where the samples were collected.

Table 1 Method performance characteristics of the high-performance liquid chromatography methods used in this study ($n = 3$ replicates)

Matrix	DON		ZEA		FB ₁		FB ₂	
	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)
Maize flour	90.4	1.2	107.2	3.0	85	15.0	76	14.0
Maize bread crust	82.7	0.8	105.9	2.4	54	20.0	26	11.0
Maize bread crumb	80.4	1.1	99.3	0.4	72	25.0	55	26.0

DON, deoxynivalenol; ZEA, zearalenone; FB₁, fumonisins B₁; FB₂, fumonisins B₂; Rec., recovery; RSD, relative standard deviation.

Mycotoxin occurrence in maize samples

The occurrence and levels of mycotoxins in the maize samples are shown in Figure 2. The results revealed that there was no relation among the provinces, concerning the toxin contamination profiles. For instance, DON occurred at a high level in one sample collected from a part of Trabzon province (sample 1), while it was not detectable in two samples from other parts of the same province. The highest levels of DON and ZEA (16100 and 1600 $\mu\text{g kg}^{-1}$, respectively) were found in the sample 1. This sample was visibly mouldy and easily distinguishable from the others as the most contaminated sample by fungi. Normally this type of sample would not be sent to market or consumed. Interestingly, this sample did not contain T-2 and HT-2, whereas the combined level of FB₁ and FB₂ (900 $\mu\text{g kg}^{-1}$) was much lower than other samples apparently healthy. This sample was used as a good source of mycotoxin contaminated maize flour to be used for preparation of contaminated bread. The other maize samples did not exceed the EU regulation limit (1750 $\mu\text{g kg}^{-1}$) established for DON in unprocessed maize (EC, 2007). No DON was detected in samples 6, 12, 13, and 15.

The incidences and levels of T-2 and HT-2 toxins in the samples were much lower than those of the other mycotoxins investigated. Only six samples (sample 2, 5, 11, 14, 19, 20) were contaminated by these toxins, all with levels < 50 $\mu\text{g kg}^{-1}$ (Figure 2b). These data will be useful in establishing EU regulation limits for these toxins in the near future since currently available data on the occurrence of these mycotoxins are not sufficient and/or reliable (Solfrizzo et al., 2009). On the other hand, ZEA levels in 15% of the samples (samples 1, 8, 20) were found to be > 350 $\mu\text{g kg}^{-1}$ (Figure 2c), the EU limit established for unprocessed maize (EC, 2007). ZEA was undetectable in the samples 13 and 15, and just above the LOQ in samples 4 and 6 (i.e. 3 $\mu\text{g kg}^{-1}$ both). All tested samples were found contaminated by FB₁ at levels between 15 and 8315 $\mu\text{g kg}^{-1}$. The levels of FB₂ were in the range from lower than LOD to 1881 $\mu\text{g kg}^{-1}$. Mean

concentrations of 1798 $\mu\text{g kg}^{-1}$ for FB₁, and 444 $\mu\text{g kg}^{-1}$ for FB₂ were observed. Combined levels of FB₁ and FB₂ exceeded 4000 $\mu\text{g kg}^{-1}$ (the EU limit for unprocessed maize) in 25% of the samples, namely samples 6, 8, 10, 11, 16 (Figure 2d). The incidence and levels of FBs reported in this study are higher than those reported by Omurtag (2001) who found FB₁ in 25.6% of the tested maize and maize-based products consumed in Turkey at levels ranging from 250 to 2660 $\mu\text{g kg}^{-1}$. When the total mycotoxin contaminations are considered, 30% of the tested samples exceeded the EU limits for at least one of these mycotoxins.

Fate of DON, ZEA and FBs during maize bread production

According to the preliminary baking experiments, bread was considered fully baked when the temperature reached 90–92 °C at the centre of the product. To be able to make a mass balance based on dry weights, both crust and crumb fractions of the breads and maize flours used for bread making were dried before analysis. Mycotoxin levels were then given on dry weight basis.

Results of the analyses of bread crumb and bread crust for DON, ZEA and FBs were compared with those obtained for maize flour used to prepare the bread. T-2 and HT-2 toxins were not considered since maize samples naturally contaminated with an acceptable level of these toxins could not be found within the 20 samples analysed. In fact the levels found in the few contaminated maize samples were below 50 $\mu\text{g kg}^{-1}$ (Figure 2). The levels of the toxins in flour and bread samples are shown in Figure 3. In general, DON, ZEA and FBs were stable during bread-making and baking process (210 °C, 60 min). In particular, mean DON levels in the flour, crust, and crumb were found to be 1937, 1922 and 1834 $\mu\text{g kg}^{-1}$, respectively. The apparent slight DON reduction observed in the crust (0.8%) and crumb (5.3%) was not statistically significant ($P > 0.05$).

The differences between the fumonisin levels of the flour and both the crust and crumb were also not statistically

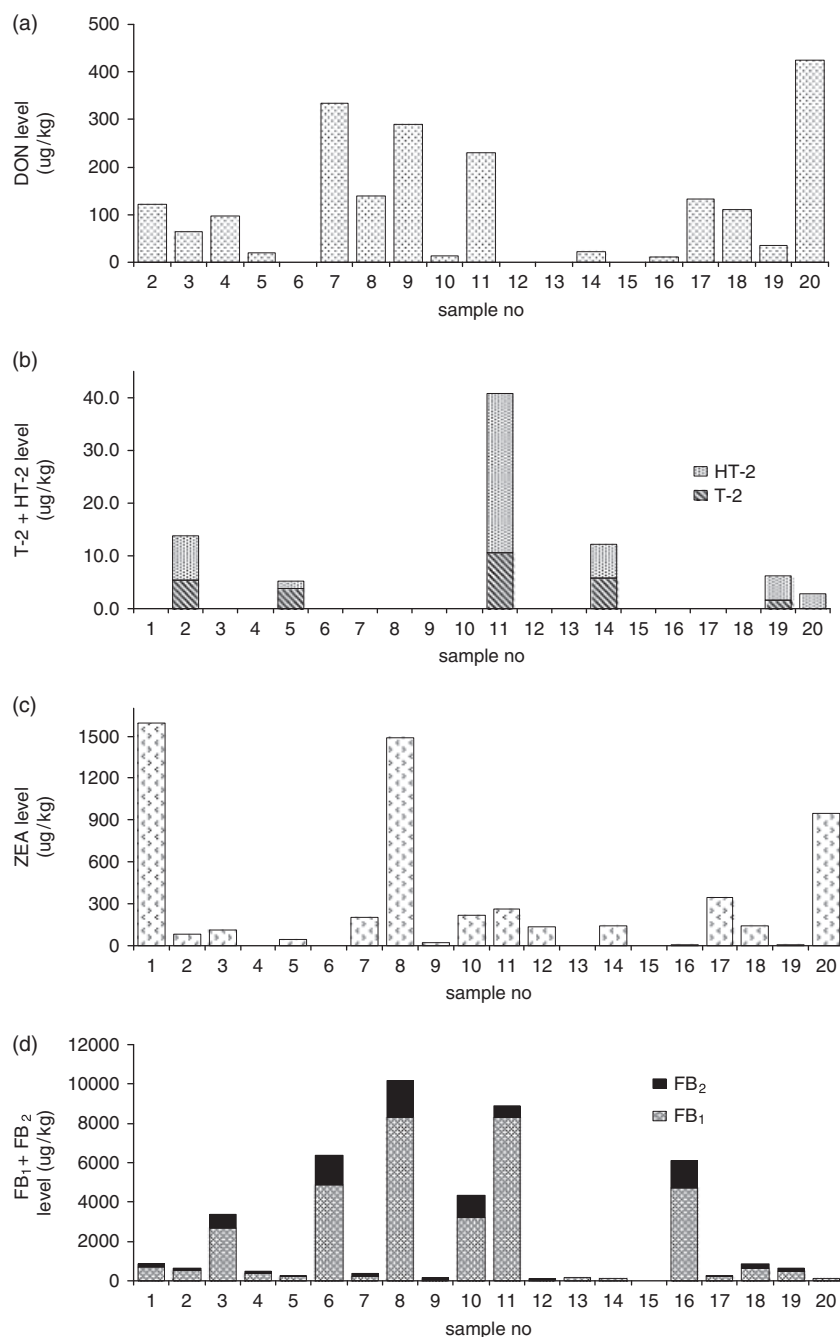


Figure 2 Levels of deoxynivalenol (DON) (a), T-2 and HT-2 (b), zearalenone (ZEA) (c) and fumonisins B₁ (FB₁), fumonisins B₂ (FB₂) (d) in the maize samples. The DON level in maize sample 1 was 16100 $\mu\text{g kg}^{-1}$.

significant ($P > 0.05$). For ZEA no significant reduction was observed in crumb whereas a statistically significant reduction was observed in crust (12.1%, $P < 0.05$). In particular, mean ZEA level in the flour was 838 $\mu\text{g kg}^{-1}$, while those in the crust and crumb of the bread were 737 and 878 $\mu\text{g kg}^{-1}$, respectively. Although the level in the crumb appears to

be higher than the starting material, the difference (4.8%) was found to be statistically insignificant ($P > 0.05$) and could be considered within the analytical variability of results.

The results of mycotoxins mass balance are reported in Table 2 and graphically shown in Figure 4. These data clearly

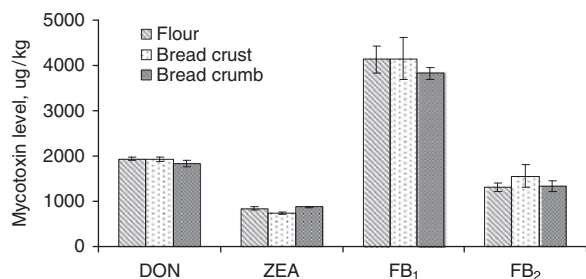


Figure 3 Stability of deoxynivalenol (DON), zearalenone (ZEA) and fumonisins B₁ (FB₁), fumonisins B₂ (FB₂) during maize bread production.

Table 2 Mass balance of mycotoxins during production of traditional Turkish maize bread

Parameters	DON	ZEA	FB ₁ +FB ₂
Weight of dry maize flour (g)	821.6		
Mycotoxin levels in dry maize flour (µg kg ⁻¹)	1937	838	5447
Mycotoxins in dry maize flour (µg)	1591	689	4475
Weight of dry crust (g)	312.1		
Mycotoxin levels in dry crust (µg kg ⁻¹)	1922	737	5710
Mycotoxins in dry crust (µg)	600	230	1782
Weight of dry crumb (g)	521.8		
Mycotoxin levels in dry crumb (µg kg ⁻¹)	1834	878	5165
Mycotoxins in dry crumb (µg)	957	458	2695
Total mycotoxins in dry crust and crumb (µg)	1557	688	4477
Mycotoxin recovered in crust with respect to the initial amount (%)	38	33	40
Mycotoxin recovered in crumb with respect to the initial amount (%)	60	66	60
Total mycotoxins recovered in crust and crumb with respect to the initial amount (%)	98	99	100

Results are corrected for recovery.

DON, deoxynivalenol; ZEA, zearalenone; FB₁+FB₂, fumonisins.

confirm the stability of the tested mycotoxins during maize bread production since 98–100% of total mycotoxins contained in the starting maize flour was recovered in bread crumb and crust.

The results of the present study for DON agree with previous studies mainly performed on wheat bread (Lancova *et al.*, 2008; Scudamore, 2008; Scudamore *et al.*, 2009). For instance, Lancova *et al.* (2008) demonstrated that baking wheat bread at 210 °C for 14 min had no significant effect on DON levels. In another study, only about 10% of DON was lost in the bread produced by the Chorleywood Bread Process, although the analytical value in the bread was

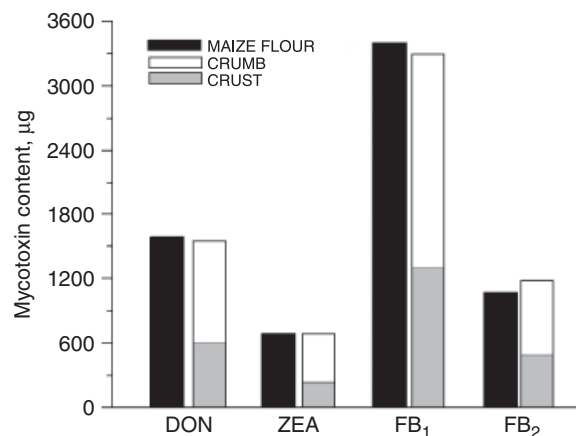


Figure 4 Total mass balance of deoxynivalenol (DON), zearalenone (ZEA), fumonisins B₁ (FB₁) and fumonisins B₂ (FB₂) (µg) during maize bread production.

about 50% of that in the flour. However, the reduction was explained by the dilution effect of increased moisture content (Scudamore, 2008).

On the other hand, Samar *et al.* (2001) found a reduction in naturally occurring DON levels during Argentinean traditional bread-making process. The maximum reduction was 56% in the Vienna bread and 41% in the French bread. The authors concluded that DON reduction during bread making might be due to both yeast fermentation and thermal decomposition of the toxin during baking. It was previously reported that the fermentation process contributes to the reduction of contamination by other mycotoxins (Westby *et al.*, 1997). Similarly, Neira *et al.* (1997) showed a significant reduction of DON during wheat bread-making process by using yeast fermentation. Thus, the stability of DON and other mycotoxins observed in the present study may be also attributed to the fact that traditional Turkish maize bread-making procedure does not involve any yeast fermentation step.

Conclusion

In conclusion, the small-scale survey on 20 maize samples has given an idea about the occurrence of mycotoxins in maize throughout Black Sea region of Turkey. The results revealed that the main *Fusarium* toxins that occur in the maize grown in this region are DON, ZEA and FBs. These mycotoxins remain stable during traditional Turkish bread making, indicating that breads produced from highly contaminated maize may create a potential risk to the population. Since no effective *Fusarium* toxin decontamination was

observed during maize bread processing, more research is needed to evaluate the effect of some other food processing methods on *Fusarium* toxins.

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References

- Bullerman L.B., Bianchini A. (2007) Stability of mycotoxins during food processing. *International Journal of Food Microbiology*, **119**, 140–146.
- Bullerman L.B., Bianchini A., Hanna M.A., Jackson L.S., Jablonski J., Ryu A.D. (2008) Reduction of fumonisin B₁ in corn grits by single-screw extrusion. *Journal of Agricultural and Food Chemistry*, **56**, 2400–2405.
- Castelo M.M., Sumner S.S., Bullerman L.B. (1998) Stability of fumonisins in thermally processed corn products. *Journal of Food Protection*, **61**, 1030–1033.
- Cazzaniga D., Basílico J.C., González R.J., Torres R.L., de Greef D.M. (2001) Mycotoxins inactivation by extrusion cooking of corn flour. *Letters in Applied Microbiology*, **33**, 144–147.
- De Girolamo A., Solfrizzo M., Visconti A. (2001) Effect of processing on fumonisin concentration in corn flakes. *Journal of Food Protection*, **64**, 701–705.
- D'Mello J.P.F., Placinta C.M., Macdonald A.M.C. (1999) *Fusarium* mycotoxins: a review of global implications for animal health, welfare and productivity. *Animal Feed Science and Technology*, **80**, 183–205.
- European Commission (EC). (2006) Commission Regulation (EC) No 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. *Official Journal of the European Union L*, **70**, 12–34.
- European Commission (EC). (2007) Commission Regulation (EC) No 1126/2007 of 28 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. *Official Journal of the European Union L*, **255**, 14–17.
- Juan C., Lino C.M., Pena A., Moltó J.C., Mañes J., Silveira I. (2007) Determination of ochratoxin A in maize bread samples by LC with fluorescence detection. *Talanta*, **73**, 246–250.
- Juan C., Pena A., Lino C., Moltó J.C., Mañes J. (2008) Levels of ochratoxin A in wheat and maize bread from the central zone of Portugal. *International Journal of Food Microbiology*, **127**, 284–289.
- Lancova K., Hajslova J., Kostelanska M., Kohoutkova J., Nedelnik J., Moravcova H., Vanova M. (2008) Fate of trichothecene mycotoxins during the processing: milling and baking. *Food Additives and Contaminants*, **25**, 650–659.
- Lino C.M., Silva L.J.G., Pena A., Fernandez M., Mañes J. (2007) Occurrence of fumonisins B₁ and B₂ in *broa*, typical Portuguese maize bread. *International Journal of Food Microbiology*, **118**, 79–82.
- MacDonald S.J., Anderson S., Brereton P., Wood R., Damant A. (2005a) Determination of zearalenone in barley, maize and wheat flour, polenta and maize-based baby food by immunoaffinity column cleanup with liquid chromatography: Interlaboratory Study. *Journal of AOAC International*, **88**, 1733–1740.
- MacDonald S.J., Chan D., Brereton P., Damant A., Wood R. (2005b) Determination of deoxynivalenol in cereals and cereal products by immunoaffinity column cleanup with liquid chromatography: Interlaboratory Study. *Journal of AOAC International*, **88**, 1197–1204.
- Neira M.S., Pacin A.M., Martínez E.J., Moltó G., Resnik S.L. (1997) The effects of bakery processing of natural deoxynivalenol contamination. *International Journal of Food Microbiology*, **37**, 21–25.
- Omurtag G.Z. (2001) Determination of fumonisins B₁ and B₂ in corn and corn-based products in Turkey by high-performance liquid chromatography. *Journal of Food Protection*, **64**, 1072–1075.
- Richard J.L. (2007) Some major mycotoxins and their mycotoxicoses – An overview. *International Journal of Food Microbiology*, **119**, 3–10.
- Ryu D., Hanna M.A., Bullerman L.B. (1999) Stability of zearalenone during extrusion of corn grits. *Journal of Food Protection*, **62**, 1482–1484.
- Samar M.M., Neira M.S., Resnik S.L., Pacin A. (2001) Effect of fermentation on naturally occurring deoxynivalenol (DON) in Argentinean bread processing technology. *Food Additives and Contaminants*, **18**, 1004–1010.
- Scudamore K.A. (2008) Fate of *Fusarium* mycotoxins in the cereal industry: recent UK studies. *World Mycotoxin Journal*, **1**, 315–323.
- Scudamore K.A., Hazel C.M., Patel S., Scriven F. (2009) Deoxynivalenol and other *Fusarium* mycotoxins in bread, cake, and biscuits produced from UK-grown wheat under commercial and pilot scale conditions. *Food Additives and Contaminants: Part A*, **26**, 1191–1198.
- Solfrizzo M., Alldrick A.J., Van Egmond H.P. (2009) The use of mycotoxin methodology in practice: a need for harmonization. *Quality Assurance and Safety of Crops & Foods*, **1**, 121–132.
- Sydenham E.V., Shephard G.S., Thiel P.G., Stockenström S., Snijman P.W., Van Schalkwyk D.J. (1996) Liquid

- chromatographic determination of fumonisins B₁, B₂ and B₃ in corn: AOAC-IUPAC Collaborative Study. *Journal of AOAC International*, **79**, 688–696.
- Visconti A., Haidukowski E.M., Pascale M., Silvestri M. (2004) Reduction of deoxynivalenol during durum wheat processing and spaghetti cooking. *Toxicology Letters*, **153**, 181–189.
- Visconti A., Lattanzio V.M.T., Pascale M., Haidukowski M. (2005) Analysis of T-2 and HT-2 toxins in cereal grains by immuno-affinity clean-up and liquid chromatography with fluorescence detection. *Journal of Chromatography A*, **1075**, 151–158.
- Westby A., Reilly A., Bainbridge Z. (1997) Review of the effect of fermentation on naturally occurring toxins. *Food Control*, **8**, 329–339.