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Fate of trichothecene mycotoxins during the processing: Milling and baking

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Abstract
Toxic secondary metabolites produced by fungi representing *Fusarium* genus are common contaminants in cereals worldwide. To estimate the dietary intake of these trichothecene mycotoxins, information on their fate during cereal processing is needed. Up-to-date techniques such as high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (LC-MS/MS) was used for the analysis of seven trichothecenes (deoxynivalenol, nivalenol, HT-2 toxin, T-2 toxin, 15- and 3-acetyldeoxynivalenol, and fusarenon-X) in bread production chain (wheat grains, intermediate products collected during milling and baking process, breads). Regardless of whether the grains were naturally infected or artificially inoculated by *Fusarium* spp. in the field, the fractions obtained from the grain-cleaning procedure contained the highest mycotoxin levels. During milling the highest concentrations of deoxynivalenol were found in the bran, the lowest in the reduction flours. Baking at 210°C for 14 min had no significant effect on deoxynivalenol levels. The rheological properties of dough measured by fermentograph, maturograph, oven rise recorder, and laboratory baking test were carried out, and based on the obtained results the influence of mycotoxin content on rheological behaviour was investigated.

Keywords: *Liquid chromatography/mass spectrometry (LC/MS), mycotoxins — trichothecenes, bakery products, bread, cereals and grain, processed foods*

Introduction

Trichothecene mycotoxins, which frequently occur in cereal grains and, consequently, in cereal-based products, are secondary metabolites produced mainly by *Fusarium* ear blight pathogens, which are widely distributed in the temperate zone of all continents (D'Mello and Macdonald 1997; Weidenbörner 2001, Creppy 2002). The incidence of these mycotoxins in food crops can vary considerably from year to year depending on many factors, weather conditions and agricultural practices being the most significant (Edwards 2004). Since these toxins can never be completely removed by processing (Kuiper-Goodman 2004), preventive measures should be adopted at all stages of the production chain (i.e. growing in the field, harvesting and storage) to prevent contamination of final food products.

Trichothecenes are highly toxic chemicals acting both at cellular and subcellular levels. Typical symptoms of *Fusarium* mycotoxicoses resulting from the consumption of seriously contaminated cereals include vomiting, dermal irritation, haemorrhagic lesions, depression of immune systems, and weight reduction (Schlatter 2004). In addition to health problems and/or economic losses, trichothecenes when present in raw materials can have negative effects on some technological processes such as during malting, brewing, fermentation, and/or baking. The physico-chemical nature of these phenomena has not yet been fully established (Hazel and Patel et al. 2004).

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To protect consumers from unacceptably high dietary intake of trichothecenes, maximum levels have been set in European Union legislation (European Commission 2006). Currently, maximum levels are regulated in cereals ($1250\,\mu g\,kg^{-1}$), flours ($750\,\mu g\,kg^{-1}$) and bread ($500\,\mu g\,kg^{-1}$) for deoxynivalenol (DON) only. Nevertheless, regulation for another two toxic trichothecenes, HT-2 toxin (HT-2) and T-2 toxin (T-2), is under preparation.

The fate of DON, the most frequent *Fusarium* toxin during various processing practices, has been documented in several studies. Experiments concerned with the distribution of DON among the milling fractions in naturally *Fusarium*-contaminated wheat showed that the highest concentration of this mycotoxin is in the bran layer; while in white flour, compared with whole grains, it is reasonably lower (Abbas et al. 1985; Tanaka et al. 1986). In another milling study (Trigo-Stockli et al. 1996) the white flour had approximately half the level of DON in the cleaned wheat, whilst the bran contained levels two or more times higher than the wheat. However, the effects of yeasts on DON levels in dough during the baking fermentation step are rather contradictory. Young et al. (1984) observed an increase of DON in yeasted products; on the other hand, a mean reduction of DON in fermented dough of over 20% was documented (Neira et al. 1997). The influence of various ingredients added to dough before baking on DON levels in bread was studied by Boyacioglu et al. (1993). Compared with control samples, a reduction of DON as high as 40% occurred in bread due to the addition of sodium bisulphite, L-cysteine and ammonium phosphate in the dough. However, other additives such as potassium bromate and L-ascorbic acid were ineffective in this context.

In addition to health risks associated with the presence of trichothecenes in the food supply, other negative aspects have to be considered. The relationship between rheological properties of dough and extent of *Fusarium* infection in wheat has been discussed by Dexter et al. (1996). Lower water absorption of flour prepared from *Fusarium*-infected grains, shorter development time, and lower stability of dough, which also became progressively sticker and difficult to handle during sheeting and moulding, were described.

The objective of the present study was to evaluate the effects of wheat processing, milling and baking on the transfer of mycotoxins into products such as flour and fermented bread. As in the case of other contaminants, the knowledge of the fate of mycotoxins during processing is important both for dietary intake estimation and adopting measures for its minimization. In addition to these aspects, the effect of mycotoxin contamination on the dough rheological properties was determined.

Materials and methods

Samples

Wheat grains. Four grain samples (each 5000 g) obtained from wheat grown under well-characterized conditions (Table I) in 2005 were supplied by the Agricultural Research Institute in Kromeriz, Czech Republic. Three of these samples (A–C) were naturally infected with *Fusarium* spp.; sample D was spray-inoculated in the field with a spore mixture of *Fusarium culmorum* and *F. graminearum* (1:1; 10^6 conidia ml^{-1} suspension; $250–300\,l\,ha^{-1}$) at the time of flowering. The levels of trichothecenes in the grains used for the processing experiments were determined by high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (LC-MS/MS) and are shown in Table I.

Processed wheat-based products

Milling fractions. Before milling with an automatic laboratory mill (model MLU-202; Bühler, Switzerland; Labofix Brabender, USA), grain samples were cleaned (sieving, scouring and polishing). During grain cleaning and screening, the waste obtained by sieving process (sieve size = 2.2 mm; the laboratory screening machine was an in-house-made prototype), outer layers of bran, and the combined waste from the scouring and polishing (a laboratory scouring in-house-made prototype; the

Table I. Characteristics of wheat grains used for processing experiments.

Sample code	Wheat cultivar	Preceding crop	Fusarium infection	Way of cultivation	Trichothecene levels ($\mu g\,kg^{-1}$)					
					DON	NIV	ADONs	Fus-X	HT-2	T-2
A	Sulamit	maize	natural	conventional	909	23	35	<1	<10	<5
B	Sulamit	trifolium	natural	organic	108	81	<10	<1	22	<5
C	Ebi	rape	natural	conventional	92	30	<10	<1	<5	<5
D	Ebi	maize	artificial	conventional	2985	<10	69	<1	<10	<5

laboratory aspirator of dust particles Labofix Brabender) were collected. Milling fractions (three break flours fractions, three reduction flours fractions, and bran fractions), according to the flow chart shown in Figure 1, were examined for their content of trichothecenes. The sizes of used sieves are shown in Figure 1. Weight proportions of wheat fractions and their moistures are reported in Table II.

Bread

The process of bread-making is outlined in Figure 2. The main components of white flour were prepared by the mixing of all three break flours and the first reduction flours obtained from grains A–D according to the schema shown in Figure 1 (for weight, see Table II). For the preparation of one batch of bread, 300 g of white flour were taken and the following

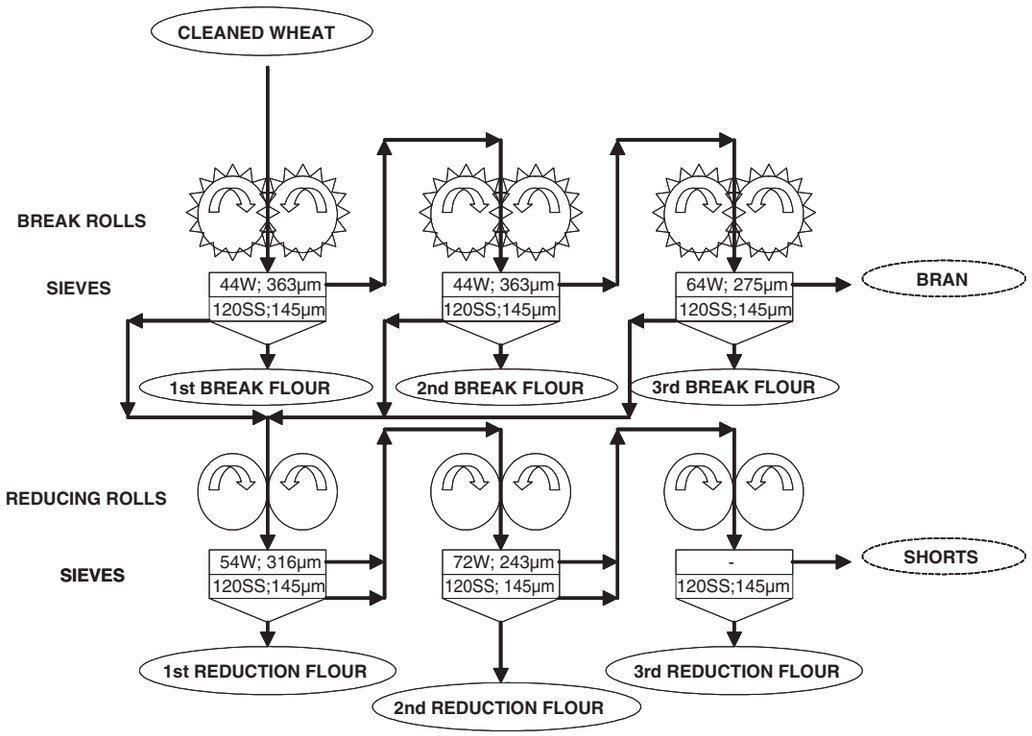


Figure 1. Simplified chart showing the milling process used for obtaining the experimental samples: W, wire; SS, stainless steel.

Table II. Characteristics of the cleaning and milling fractions.

		Weight proportion of wheat fractions (%)/moisture (%, w/w)			
		Sample code (see Table I)			
Process	Fraction	A	B	C	D
Cleaning	Uncleaned grain	100.0/13.9	100/13.3	100.0/13.5	100.0/13.6
	Screening	3.0/13.6	8.8/12.8	5.9/13.1	7.8/13.1
	Outer layer of bran	0.5/13.9	0.5/13.0	1.0/13.2	0.7/13.3
	Cleaned grain	85.1/14.0	87.7/13.1	92.7/13.3	87.4/13.5
Milling	Cleaned grain	100.0/14.0	100.0/13.1	100.0/13.3	100.0/13.5
	Bran	19.7/14.2	25.1/13.4	19.4/14.4	22.0/14.5
	Shorts	14.0/*	5.5/*	9.8/*	10.1/*
	First reduction flour	5.8/13.4	4.9/13.1	9.1/14.4	7.9/12.4
	Second reduction flour	6.5/13.2	7.5/13.2	9.9/14.2	8.1/14.3
	Third reduction flour	1.8/12.4	1.8/12.3	2.4/12.4	1.9/13.0
	First break flour	27.7/13.6	27.7/13.3	29.4/13.9	36.5/11.7
	Second break flour	11.3/13.1	11.3/13.2	11.8/13.2	10.2/14.5
	Third break flour	2.9/12.5	2.9/12.5	2.8/12.0	2.4/14.2

*Shorts were not available for analysis.

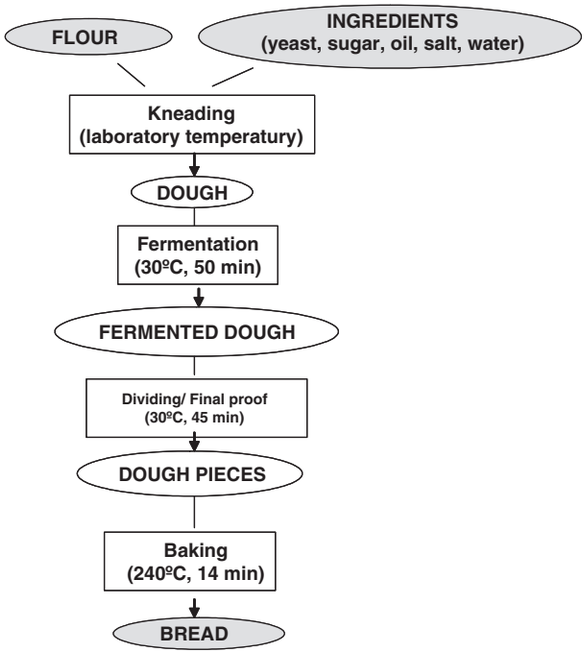


Figure 2. Bread-making process.

ingredients added: Yeast *Saccharomyces cerevisiae* (12 g), saccharose (5.1 g), vegetable oil (4.5 g), salt (2.1 g), and distilled water (150–165 ml; with the volume varying according to bonding power). Immediately after kneading the dough was placed in a covered bin (container) containing a laboratory thermostat and allowed to ferment at 30°C for 50 min. The dough was then divided into pieces of approximately 70 g each and moulded by hand. The final proof of the dough pieces proceeded at the same temperature (30°C) as during the stage of fermentation for 45 min. The loaves of bread were baked in an electric laboratory oven at 240°C for 14 min. All intermediate products prepared within the baking process were dried for 24 h at 40°C before analysis.

Evaluation of rheological properties

To describe the visco-elastic behaviour of dough during kneading, fermentation and proofing, the determination of the quality of the dough was carried out by rheological instruments such as a fermentograph (SJA, Sweden), maturograph, oven rise recorder (both Brabender, Germany) and laboratory oven (Zaklad Badawczy Przemyslu Piekarskiego, Poland). The fermentograph monitors the first stage of dough proofing, called fermentation, by measuring dough volume and fermentation gasses. The maturograph is used to record the fermentation process during the proofing time by means of a sensing probe which touches the dough surface. The oven rise recorder describes the changes in volume of dough during the first stage of baking in an oil bath in a temperature range from 25 to 100°C when changes of proteins and starch caused by their denaturation are occurring in the dough during baking.

Trichothecene analysis

LC-MS/MS was used for the analysis of seven trichothecene mycotoxins in sample extracts purified by solid-phase extraction (SPE). Wheat grains (raw materials), milling fractions and intermediates were collected during baking as well as the resulting breads.

Standards

Trichothecene standards (deoxynivalenol, DON; nivalenol NIV; HT-2 toxin, HT-2; T-2 toxin, T-2; 15- and 3-acetyldeoxynivalenol, ADONs; and fusarenon-X, Fus-X) were purchased from Sigma-Aldrich (Germany) and Biopure (Austria). Certified reference materials, 'DON in wheat flour' ($<0.05\,mg\,kg^{-1}$, BCR 396, Belgium) and 'DON in naturally contaminated wheat' ($0.7 \pm 0.1\,mg\,kg^{-1}$, R-Biopharm Rhone, UK), were used as controls to ensure the accuracy of measurements. The values obtained for DON were in the uncertainly range of certificate concentrations.

Extraction and clean-up procedure

Representative samples (12.5 g) were extracted with 50 ml acetonitrile–water mixture (84:16,v/v) for 1 h using an automatic shaker (IKA Laboratortechnik, Germany). Crude extracts were then filtered (Filtrak No. 390, VEB Freiberger, Germany) and 8-ml aliquots transferred into sample tubes to which 80 µl of acetic acid (99%; Sigma-Aldrich) were added. Purification was achieved by SPE employing the MycoSep^{TM} 226 cartridges (Romer, Austria). A total of 4 ml of purified extract were evaporated to dryness and the residues transferred into 1 ml of a water–methanol mixture (80:20, v/v) and passed through a 0.2 µm microfilter (Alltech, USA) before further analysis.

Chromatographic conditions

High-performance liquid chromatography (HP1100 Binary Series LC system; Agilent Technologies, USA) coupled with a mass spectrometer (Finnigan LCQ Deca, USA) was used for the analysis of purified extracts. Chromatographic separation of sample components was carried out on a reverse-phase column with polar endcapping (Synergi Hydro RP, 150 × 3 mm × 4 µm' Phenomenex, USA) heated at 40°C and operated under gradient conditions.

Table III. Validation data obtained by liquid chromatography coupled with tandem mass spectrometry detection (LC-MS/MS) method ($n = 5$).

Parameter	Matrix	DON	NIV	Fus-X	ADONs	HT-2	T-2
LOD ($\mu g\,kg^{-1}$)	Wheat dough bread	0.5	1	1	1	5	0.5
LOQ ($\mu g\,kg^{-1}$)		5	10	10	10	10	5
Recovery* $\pm$ RSD (%)	wheat	87.6 ± 3.6	53.3 ± 3.5	83.1 ± 4.5	82.8 ± 5.9	95.4 ± 3.2	83.5 ± 4.7
	dough	89.1 ± 3.1	42.8 ± 9.7	80.8 ± 7.1	84.0 ± 6.5	85.9 ± 6.0	91.2 ± 4.0
	fermented dough	87.4 ± 4.1	46.7 ± 6.7	83.8 ± 4.9	85.2 ± 5.4	88.2 ± 5.4	92.0 ± 3.8
	dough after proof	87.4 ± 4.1	46.7 ± 6.7	83.8 ± 4.9	85.2 ± 5.4	88.2 ± 5.4	92.0 ± 3.8
	bread	86.4 ± 2.7	43.8 ± 5.2	85.1 ± 5.6	86.3 ± 6.0	86.1 ± 3.9	89.0 ± 4.1

*Analytes' levels in spiked samples were $480\,\mu g\,kg^{-1}$.
LOD, limit of detection; LOQ, limit of quantitation; RSD, relative standard deviation.

The mobile phase was composed of 10 mM ammonium acetate in purified water (A) and methanol (B). The flow rate of the mobile phase was set to $0.5\,ml\,min^{-1}$ and the injection volume was 20 µl. Gradient elution was performed starting from A:B (80:20, v/v) and reaching A:B (30:70, v/v) in 8 min. From 8 to 15 min the ratio A:B (30:70, v/v) was stable and then jumped to A:B (80:20, v:v). The time of post run lasted 7 min.

Identification and quantification of analytes was performed using MS/MS with the following parameters (ion source type, APCI operated both in negative- and positive-ion modes; capillary temperature, 150°C; vaporizer temperature, 450°C; nitrogen sheath gas flow, $1.2\,l\,min^{-1}$; nitrogen auxiliary gas flow, $3\,l\,min^{-1}$; source voltage, 6 kV; collision gas, helium; scan type, selected reaction monitoring). APCI ionization modes ($\pm$) and monitored fragments *m/z* (parent ion > daughter ion, confirmation ion) used for individual analytes were DON^- (371 > 311, 281), NIV^- (355 > 295, 265), $FUS\text{-}X^-$ (413 > 353, 263), $ADONs^-$ (397 > 337, 307), $HT\text{-}2^+$ (442 > 425, 263) and $T\text{-}2^+$ (317 > 273, 299).

Performance characteristics of analytical method/quality assurance

Limits of detection (LODs) and limits of quantification (LOQs), recoveries and repeatabilities expressed as relative standard deviations (RSDs) which were obtained within the validation process are reported in Table III. Calibration curves for all analytes were linear within the working range from 5 to $10\,000\,\mu g\,kg^{-1}$. Squared correlation coefficients (R^2) were in the range 0.9991–0.9999 for 11-point calibration curves.

The analytical method used for samples examination was accredited (ISO 17025) for cereals; as a part of external quality control the trueness of generated data was demonstrated through participation in the Food Analysis Performance Assessment Scheme (FAPAS) organized by the Central Science Laboratory (CSL, York, UK). The *z*-scores for all analysis (DON, ZON, HT-2 and T-2) were in the range ± 2.

Moisture determination

Moisture was determined gravimetrically by drying samples in an oven for 2 h at 131 ± 2°C according to ISO Standard No. 712 'Moisture determination in cereals and cereal products'.

Results and discussion

In common with some other contaminants, the levels of mycotoxins in processed foods can differ considerably from those in raw materials. Depending on the household and/or industrial processing practised, both reduction and accumulation of toxins can occur in a particular product. In any case, these changes should be taken into account when estimating consumer exposure on the basic of data obtained by analysis of cereal grains.

Distribution of Fusarium mycotoxins in wheat grains

For processing experiments, four wheat grain samples, representing various contamination characteristics (both in terms of total content of trichothecenes and their patterns), were used. A fairly uneven distribution of trichothecenes within the fractions obtained during the milling process was apparent, and is shown in Table IV. Relatively very high contents of trichothecenes were found in waste fractions obtained within cleaning process. Effective removing of all screenings and outer layers of bran from the surface of grains during cleaning steps reduced the DON content by 50, 55, 41, and 47% in cleaned wheat samples A, B, C, and D, respectively. The contents of DON in individual milling fractions are shown in Figure 3. Considering the weight contribution of various flour fractions to the total mass of cleaned grains taken for milling, most of the

Table IV. Distribution of trichothecene mycotoxins in milling fractions.

	Sample code (see Table I)																			
	A					B					C					D				
	Mycotoxin content (μg kg^{-1} dry weight)																			
Product	NIV	DON	ADONs	HT-2	T-2	NIV	DON	ADONs	HT-2	T-2	NIV	DON	ADONs	HT-2	T-2	NIV	DON	ADONs	HT-2	T-2
Uncleaned grain	23	909	35	<LOQ	<LOQ	81	108	<LOQ	29	<LOQ	30	92	<LOQ	<LOD	<LOD	<LOQ	2985	69	<LOQ	<LOQ
Impurity	19	1665	43	173	26	44	705	<LOQ	23	<LOQ	88	105	43	<LOD	<LOD	22	8419	139	32	11
Dust	36	7175	94	145	9	245	2689	49	226	36	42	671	43	21	<LOD	69	25020	425	37	<LOD
Cleaned grain	<LOD	456	<LOD	<LOQ	<LOD	<LOD	59	<LOD	<LOQ	<LOQ	16	38	<LOD	<LOD	<LOD	<LOD	1413	12	<LOD	<LOD
First break flour	<LOD	391	<LOD	<LOD	<LOD	<LOD	64	<LOD	<LOD	<LOD	<LOD	42	<LOD	8	<LOD	<LOD	1057	<LOD	<LOD	<LOD
Second break flour	<LOD	278	<LOD	<LOD	<LOD	<LOD	42	<LOD	<LOD	<LOD	<LOD	34	<LOD	<LOD	<LOD	<LOD	827	<LOD	<LOD	<LOD
Third break flour	<LOD	270	<LOD	<LOD	<LOD	<LOD	49	<LOD	<LOD	<LOD	<LOD	39	<LOD	<LOD	<LOD	<LOD	786	<LOD	<LOD	<LOD
First reduction flour	<LOD	314	<LOD	<LOD	<LOD	<LOD	32	<LOD	<LOD	<LOD	<LOD	27	<LOD	<LOD	<LOD	<LOD	764	<LOD	<LOD	<LOD
Second reduction flour	<LOD	202	<LOD	<LOD	<LOD	<LOD	26	<LOD	<LOD	<LOD	<LOD	18	<LOD	<LOD	<LOD	<LOD	455	<LOD	<LOD	<LOD
Third reduction flour	<LOD	170	<LOD	<LOD	<LOD	<LOD	33	<LOD	<LOD	<LOD	<LOD	23	<LOD	21	<LOQ	<LOD	396	<LOD	<LOD	<LOD
Bran	<LOD	975	<LOD	78	<LOD	<LOD	117	<LOD	93	77	62	90	<LOD	<LOD	<LOD	<LOD	2451	32	<LOD	<LOD

LOD, limit of detection; LOQ, limit of quantitation.

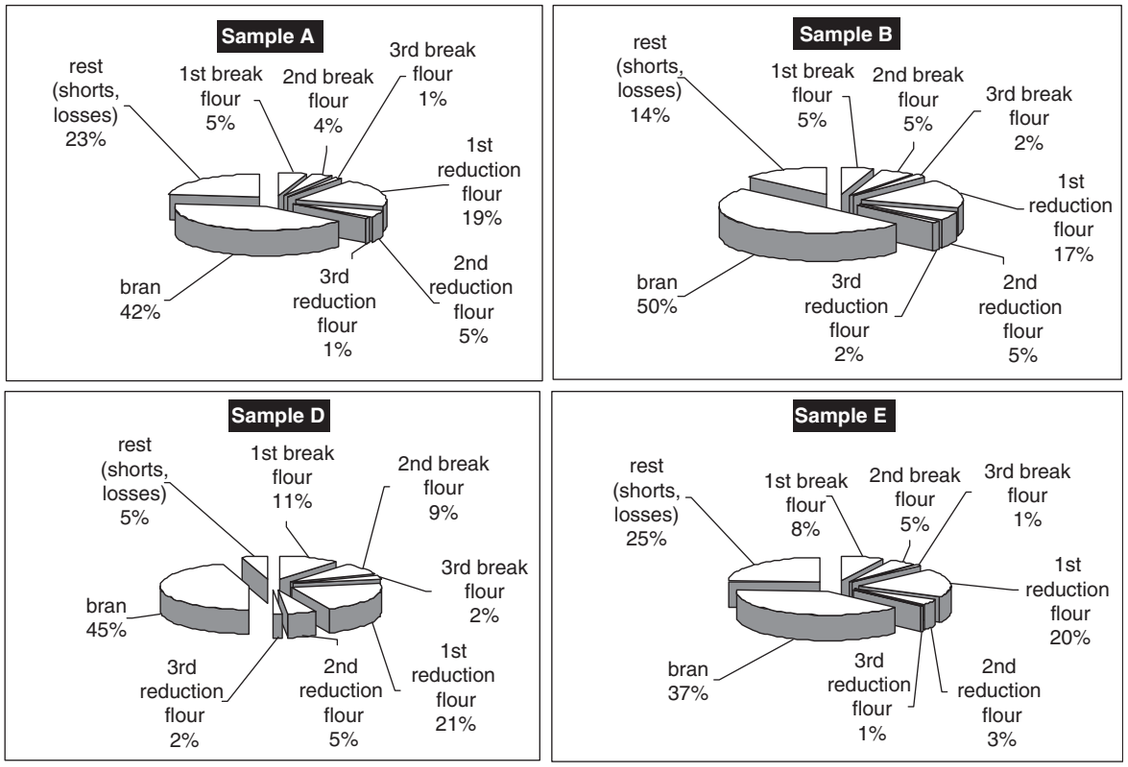
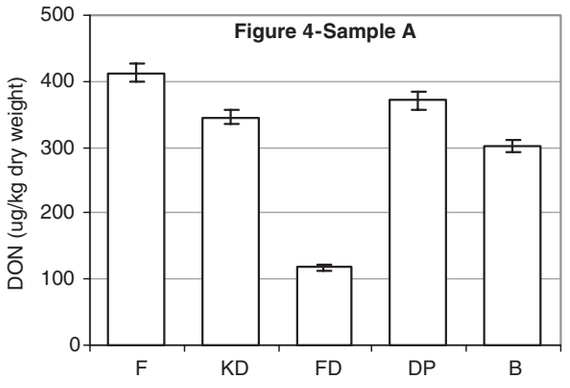


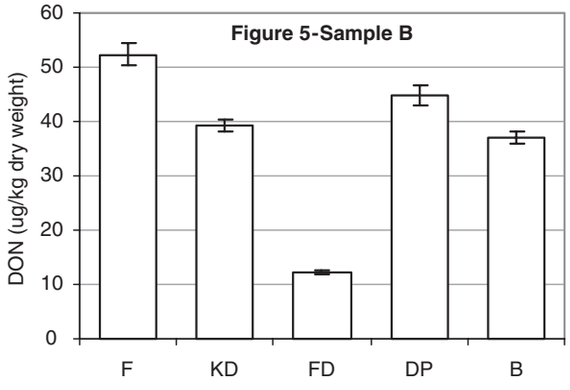
Figure 3. Deoxynivalenol (DON) distribution (%) in milling fractions (the total DON amount in of 4000 g of cleaned grains = 100%; the contributions were calculated on the weight of obtained milling fractions shown in Table II) and determination of the concentration levels of trichothecenes in these samples summarized in Table I.

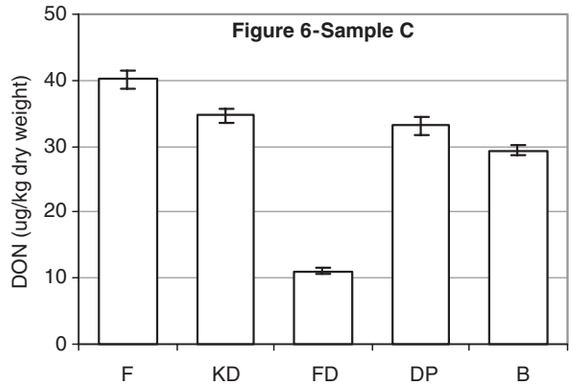
DON (19, 17, 21, and 20% in A, B, C, and D samples, respectively) was contained in the first reduction flours because of high DON levels and the amount of this type of milling fraction. Very similar results were reported by Japanese researchers (Tanaka et al. 1986) who used the same type of laboratory mill for fractionation of naturally contaminated wheat. Generally, the data obtained by a different mill might not be comparable.

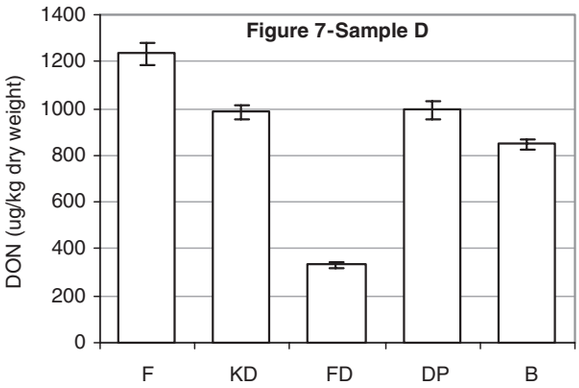
Changes of DON concentration during baking process

Four white flours prepared by mixing respective break flours and the first reduction flours (Figure 1) were used for baking experiments. DON levels in these flours corresponding to grains A, B, C, and D were 413, 52, 40, and 1223 $\mu g\,kg^{-1}$, respectively. Other target trichothecene mycotoxins (NIV, ADONs, Fus-X, HT-2, and T-2) were not detected in any of the flour samples. The changes in DON concentrations during dough processing (kneaded, fermented, and final proof dough) and in the final bread are illustrated in Figures 4–7 and summarized in Table V. Considering the total amount of DON contained in white flours which were taken for breads production, its levels were higher by 21–40% in kneaded dough. However, during fermentation a significant decrease occurred of approximately 38–46% of the original content (the calculation, i.e. basis for flour amount in the same in all these products). Surprisingly the dough pieces at the end of proofing phase contained relatively high DON levels ranging from 132 to 145% of the total flour content in products A, B, C, and D. However, when comparing the content of DON in the raw materials (flours) and final products (loaves), practically no changes occurred with a maximum loss of 6% in bread relevant to sample D. In spite of apparently unaffected DON levels in the dough processing is accompanied with distinct fluctuation of its contents. Release of DON from its conjugated form is probably followed by its transformation into a substance that cannot be determined by conventional analysis. Unfortunately, its nature remains unclear. It is worth noting that the same profile of changes in DON levels occurred in all samples regardless of the extent of contamination. Remarkably, a temporary drop of DON (approximately 40%) as the result of dough fermentation was also observed in earlier studies. Overall, the DON content in loaves was practically the same as that









Figures 4–7. Deoxynivalenol (DON) levels in intermediate products collected during baking process: white flour, F; kneaded dough, KD; fermented dough, FD; dough after final proof, DP; bread, B. Concentrations are expressed as the dry weight of the respective product.

Table V. Effect of baking process on deoxynivalenol (DON) levels; content of DON in flour taken for processing = 100%.

	Sample Code (see Table I)			
	Percentage of DON			
Product	A	B	C	D
Kneaded dough	135	121	140	131
Fermented dough	46	38	44	44
Dough after final proof	145	139	134	132
Bakery product	100	96	99	94

determined in white flours taken for their preparation. In other words, no mitigation of this mycotoxin occurred during the bread-making process. This conclusion complies with results obtained in several studies carried out in various countries (Table VI). Recently, many discussions on masked mycotoxins in cereals have been started. Deoxynivalenol 3-glucoside has been shown to be the most abundant DON derivative (Berthiller et al. 2005). Unfortunately, it was beyond the scope of the present study to search for DON conjugates. In any case, the efficiency of extraction process was tested for each baking intermediates. Hence, it is unprovable that drops of DON content were due to its bounding by the yeast.

Effect of mycotoxin content on rheological properties

As mentioned above, besides the health risk associated with infection of cereals by *Fusarium* fungi, other problems corresponding to the quality of flour can be encountered. Two important fermentographic characteristics — dough volume and time of fermentation — describe the properties and behaviour of dough before the collapse of its structure caused by biochemical changes within fermentation. The values of the two fermentographic factors mentioned above in wheat samples A, B, and D were higher by approximately 40% than in sample C (the lowest DON level) (Table VII).

Maturograph characteristics belong to the most important parameter in a description of rheological dough properties. Proofing time ensures the optimum time for reaching the maximum volume of final bakery products. It was clear that dough prepared from infected grains needed a longer period to approach this optimal time. Dough resistance against mechanical stress during proofing correlated

Table VI. Deoxynivalenol (DON) transfer from contaminated flour to bread (reported in earlier published studies).

Sample characteristics	Product/process conditions	DON in flour (μg kg^{-1})	Remaining DON in bakery product (% of flour)*	References
Naturally infected wheat	non-yeasted sponge, 170°C for 30 min	193	105	Tanaka et al. (1986)
Spiked flour (500 μg kg^{-1})	non-yeasted sponge, 170°C for 30 min	665	101	Tanaka et al. (1986)
Artificially infected wheat heads	yeasted bread, 220°C for 20 min	3130	93	Boyacioglu et al. (1993)
Naturally infected wheat	yeasted bread, 210°C for 10 to 40 min	500–1000	24–100	Neira et al. (1997)
Naturally infected wheat	yeasted bread, 205°C for 30 min	4100	107	Scott et al. (1983)
Naturally infected wheat	yeasted bread, data not available	520.9	31–81	Abbas et al. (1985)

*DON in flour (100%).

Table VII. Rheological characteristics of fermented dough.

	Fermentograph		Maturograph			Oven rise recorder	
Sample code	V_D (FeU)	T_{FER} (min)	T_{MAT} (min)	R_D (MaU)	S_{MAT} (min)	V_B (OU)	Oven rise (OrU)
A	91	60	40	820	6	620	190
B	83	57	46	725	8	490	190
C	61	44	34	715	12	480	195
D	85	59	40	880	6	595	220

FeU, fermentographic unit; MaU, maturographic unit; OrU, oven rise recorder unit; V_D, dough volume; T_{FER}, fermentation time; T_{MAT}, proofing time; R_D, dough resistance; S_{MAT}, proofing stability; V_B, bread volume.

DON content with the higher contamination in dough giving the higher dough resistance. On the other hand, the elasticity of fermented dough was not affected by the extent of contamination. The proofing stability of dough reflects the time tolerance of optimal proofing and so ensures the highest volume of the final products. Regarding this parameter, the dough stability prepared from the sample contaminated with the lower amount of DON (sample C) was twice as high in comparison with the flour contaminated with the most DON (sample D).

The baking volumes recorded by an oven rise recorder, which indicates the final volume of baked products, were slightly higher for samples containing mycotoxins. This trend was also found for another rheological factor: Oven rise that characterizes the difference in volume between a baked product and the unprocessed dough.

Conclusions

The results obtained in this study can be summarized as follows:

- The highest levels of both type B trichothecenes (deoxynivalenol, nivalenol, sum acetyl-DON) and type A trichothecenes (HT-2 and T-2 toxin) were concentrated in waste fractions (screenings, outer layers of bran) obtained during cleaning of wheat grains. On this occasion a potential health risk for staff breathing these materials at gristmills should be mentioned.
- A substantial part of DON in cleaned grains is located in the bran. Nevertheless, approximately 40% of its original content was still left in flour fractions; the first break flour was the most contaminated.
- Pronounced changes of DON levels in dough samples taken in particular steps of baking process might be due to transformation of DON forms (free/conjugate). More research is needed to explain these phenomena.
- No significant reduction of free DON levels occurred as the result of bread-baking process (210°C, 14 min).
- Some rheological properties such as proofing time and dough stability were found to be worse in samples prepared from more DON-contaminated grains. Nevertheless, more than DON level, the overall impact of the mould growth on the rheological properties is important.

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