

Mycotoxin survey of wheat samples graded according to their technological quality

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Wheat grain samples from 108 fields in Slovakia were analysed for their technological quality parameters and deoxynivalenol (DON) content. A total of 206 samples were sorted into those that were suitable (S; n = 186) and those that were unsuitable for human consumption (U; n = 20). The S samples were sorted into grain quality grades (E – elite; A – standard; B – minimum; P – biscuit). The natural occurrences of DON were 72.3 % in the E; 84.9 % in the A; 86.1 % in the B; 58.1 % in the P and 100.0 % in the U samples. The mean DON content was 0.55 mg kg⁻¹ in the E; 0.47 mg kg⁻¹ in the A; 0.67 mg kg⁻¹ in the B; 0.36 mg kg⁻¹ in the P and 1.67 mg kg⁻¹ in the U samples. The natural mean DON contamination of the human consumption samples was lower (0.52 mg kg⁻¹) than for the samples that were unsuitable for human consumption (1.67 mg kg⁻¹).

Key words: *Triticum aestivum* L., grains, technological parameters, deoxynivalenol

Introduction

Wheat was the most important cereal commodity harvested in Europe in 2010 and covered an agricultural area of more than 56 million ha. In 2011 it had reached more than 59 million ha. In Slovakia (located in Central Europe), wheat is grown on 49.9 % of the area where cereals are cultivated. If cereal grains meet certain technological standards, then the grains can be used in food production. If they do not, then they are used in feed. The grain grades are a tool that is used to define the quality of wheat grain used for human consumption. The wheat (*Triticum aestivum* L.) quality requirements for human consumption purposes are specified in Slovak Technical Standard 46 1100-2:2003-07 (STS 2003). This standard divides wheat into four grain grades. Three of them are for baked goods and one for biscuit goods: E – elite quality, A – standard quality, B – minimum quality requirements for the intervention purchase of wheat and P – biscuit quality. The technological parameters assessed are: moisture (M), weight volume (Wv), protein content (PC), wet gluten (WG), gluten index (GI); sedimentation index according to Zeleny (SI), and falling number (FN).

Contaminant level is another criterion used to decide whether wheat grains are suitable for food production. Mycotoxins produced by fungi are dangerous contaminants of wheat grain. Grains in spikes can be contaminated during vegetative growth by so-called field mycotoxins produced by *Fusarium* spp. Many studies have shown that wheat grains attacked by *Fusarium* spp. can be contaminated by one or more mycotoxins, as well as by so-called masked mycotoxins (i.e. mycotoxin conjugates) (Placinta et al. 1999, Berthiller et al. 2013). Research concerning natural occurrence of mycotoxins in Europe has shown that deoxynivalenol (DON) is one of the most frequently found mycotoxins in foodstuffs (Schollenberger et al. 2005), in feed (Schothorst 2004, Streit et al. 2013), in cereal products and in feed mixtures (Cegielska-Radziejewska et al. 2013) and its occurrence is also considered to be an indicator of the possible presence of other, more toxic, trichothecenes (Jajic et al. 2008). Analyses of cereal products, processed cereal products and wheat-based baby food collected from retailers and farms in Slovakia have confirmed that, of the analyzed *Fusarium* mycotoxins, DON was the most prevalent in the samples (Belajová et al. 2010). Van der Fels-Klers et al. (2012) suggested that climate changes would increase deoxynivalenol contamination of wheat in northwestern Europe because deoxynivalenol contamination had increased by up to three times in most of the studied regions in northwestern Europe over the past few years.

Arunachalam and Doohan (2013) reported the various toxicological effects of trichothecenes at the cellular and molecular level in humans, animals and plants. The effect of DON alone and in combination with fumonisin B1 was evaluated by Basso et al. (2013) using histological, immunohistochemical and ultrastructural assays of jejunal explants from piglets. They found that DON alone or in combination induces significant damage to intestinal tissue.

DON and its acetylated derivatives, 3-acetyldeoxynivalenol (3 ADON) and 15-acetyldeoxynivalenol (15 ADON), are mainly produced by *Fusarium culmorum* and *F. graminearum* (Bottalico et al. 1998). Chemotype characterisation of *F. graminearum* and *F. culmorum* isolates from Central and Southern Europe frequently showed that they were chemotype 15 ADON (Stepień et al. 2008, Talas et al. 2011) and from Northern Europe they were chemotype 3 ADON (Yli-Mattila 2010). The amount of natural contamination by this mycotoxin in wheat samples is influenced by numerous factors (environmental factors, plant growth stage, inoculum dosage, pathogen aggressiveness, chemotype, lodging, tillage system, cultivar resistance and fungicide application), reported Wegulo (2012). A close correlation between *Fusarium* damage to spikes, *Fusarium* damage to kernels (FDK), *Fusarium* protein equivalents (FPE) in kernels and DON content in wheat kernels (Šliková et al. 2009) was determined in experiments where spikes were artificially infected with *Fusarium* spp.

It is generally accepted that *Fusarium* infection leads to grain weight losses and this has an impact on some technological traits. Papoušková et al. (2011) reported that *Fusarium* damage had a detrimental effect on the technological and baking quality of wheat. Previous studies have analysed flour from wheat grain samples obtained from experiments that had artificially infected grain with *Fusarium* spp. but the results were not consistent. Prange et al. (2005) reported that high *Fusarium* infection levels and high DON contents did not necessarily reduce baking quality and this finding was independent of infection time. Several authors have shown that high DON content caused poor flour color, a decline in wet gluten content (Dexter et al. 1996), no reduction in protein content and reductions in Zeleny sedimentation and falling number (Capouchová et al. 2012). Some authors also suggested that there were reductions in dough functionality and loaf volume potential (Nightingale et al. 1999) and an increase in loaf shape deformation (Wang et al. 2005). Gärtner et al. (2008) found that the effects of *Fusarium culmorum* infection on baking quality parameters, water absorption, dough softening and dough resistance differed between susceptible varieties and resistant varieties.

The aims of this study were (1) to monitor the technological quality of wheat samples collected from maize production regions; (2) to determine the potential of natural contamination of wheat samples suitable for human consumption by mycotoxin DON content; (3) to compare the sample contamination levels in the different wheat quality grades and (4) to investigate whether samples containing excessive amounts of DON occur in other wheat samples that were graded as being suitable for human consumption.

Material and Methods

Samples

Mature wheat grains were collected during the 2010 and 2011 growing seasons so that their technological parameters and DON content could be measured. A total of 206 samples were provided directly by growers. Each sample (2000 g) was collected from fields in Slovakia (Fig. 1 and Fig. 2) that were located in a maize producing region where the elevation above sea level is 112–200 meters; average annual temperature is 9–10 °C and annual rainfall is above 600 mm. The soils in the region are black and, in particular, brown soils. The growers used perennial fodder crops, legumes, mixtures of legumes and cereals, rapeseed and maize for silage as preceding crops. The several individual samples were taken from one collection place (from particular variety). The total average sample with the weight of 2000 g was divided into 2 parts (1000 g to determine technological parameters and 1000 g to determine DON content). After grinding the full lot sample, a subsample was taken for analysis.

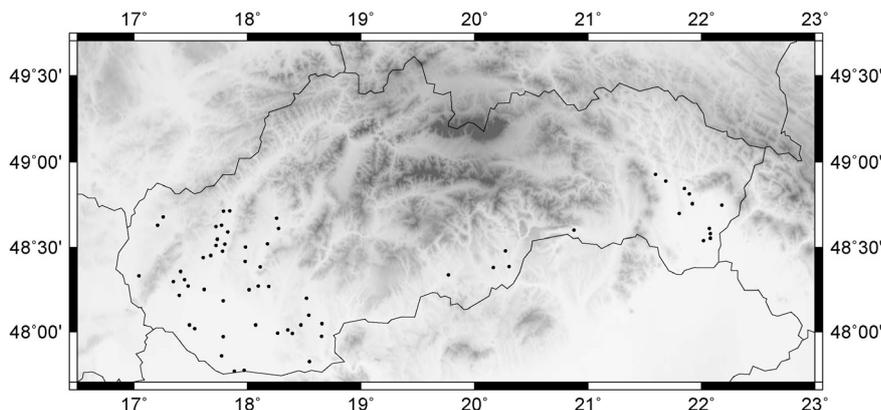


Fig. 1. Field locations in the Slovak Republic from which wheat samples were collected in 2010 (n = 62).

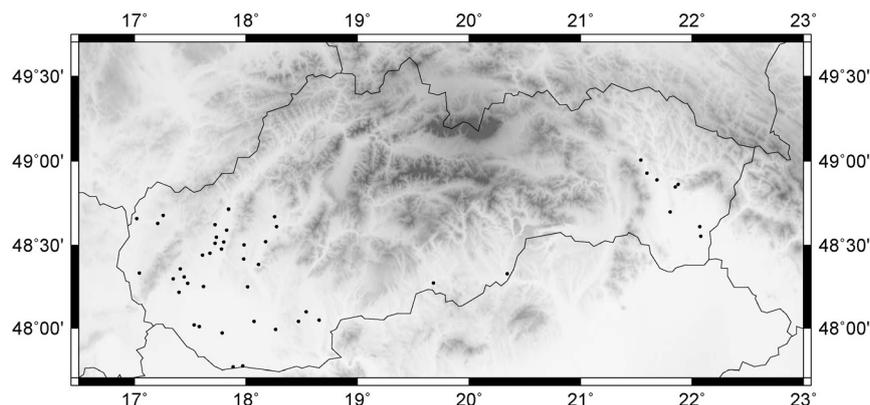


Fig. 2. Field locations in the Slovak Republic from which wheat samples were collected in 2011 (n = 46).

Technological parameter analyses

The weight volume was determined according to EN ISO 7971-3 (GAC®500 XT, DICKEY-John Europe S.A.S, France). The nitrogen content was determined according to Dumas (TruMac N, LECO Corporation, St. Joseph, MI). A conversion factor of 5.7 was used to calculate the protein content from the nitrogen content (AACC 46–30.01). The wet gluten content and the gluten index were determined according to ICC Standard No.155 (Glutomatic 2200, Perten Instrumenst, Sweden). The gluten index (GI) is a method of analysing wheat protein that provides a simultaneous determination of gluten quality and quantity. The GI value expresses a weight percentage of the wet gluten remaining on a sieve after automatic washing in salt solution and centrifugation (Centrifuge 2015, Perten Instrumenst, Sweden). GI allows a reliable prediction of bread making quality by means of the Zeleny sedimentation index, which was determined according to ISO 5529 (Shaker Brabender, Germany). The falling number was determined according to ISO 3093 (Falling Number 1800, Perten Instruments, Sweden). All parameters were analysed in duplicate.

Slovak technical standards (STS) 46 1100-2 for cereals destined for human consumption are described in Table 1.

Table 1. Grades of wheat quality and minimum values of parameters according Slovak Technical Standard (STN 46 1100-2, 2003).

Parameter	Grade			
	E	A	B	P
Moisture (%)	14	14	14	14
Weight volume (g.l ⁻¹), at least	780	760	730	750
Nitrogen content (N x 5,70) in dry mass (%), at least	12.5	11.5	10.5	9.5
Falling number in groat of 7g (s), at least	220	220	220	160
Sedimentation index according Zeleny (ml), at least	30	25	22	–
Recommended quality trait				
Wet gluten content in dry mass (test G _w) (%), at least	27	25	23	20

E - elite quality, A - standard quality, B - minimum quality requirements for intervention purchase of wheat, P – biscuit quality

Deoxynivalenol

A commercial ELISA kit was used to determine the DON concentration in the wheat samples (Ridascreen Fast DON, RBIopharm, Darmstadt, Germany) with limit of detection < 0.2 mg.kg⁻¹ (ppm) and limit of quantification 0.2 mg.kg⁻¹ (ppm). The grain samples were ground (Ultra Centrifugal Mill, type ZM 100, Retsch, Haan, Germany) with sieve size 1.00 mm. Then 100 ml of distilled water was added to 5 g of each sample and the mixture filtered. The filtrate, in aliquots of 50 µl, was used for analysis. The absorbencies of the wells were determined photometrically at 450 nm (MRX II, Dynex Technologies, Chantilly, Virginia, USA) and the DON concentrations were calculated in mg•kg⁻¹ by Revelation Version 4.25 (Dynex Technologies). Statistical analysis was performed using SPSS software 11.5 (SPSS, Chicago, Illinois, USA) and the statistical significance levels were set at 95 % (p < 0.05) and 99 % (p < 0.01).

Results and discussion

Technological parameter analyses of the wheat samples

Table 2 shows the results from the technological parameter analyses for all the wheat samples (n = 206). ANOVA analysis of M, Wv, PC, WG, GI and FN showed that there were significant differences between the years, except for Zeleny sedimentation index (ZS) where there was no significant difference, which showed that this parameter was the least affected by the weather (Table 2). The average values for the technological parameters showed that all the samples reached grade E according to the STS criteria (2003, Table 1) in 2010, but in 2011 the PC parameter only reached grade A (Table 2). The quality of the analyzed samples was higher in 2010 than in 2011. Around 9.7 % of the samples did not meet the S grade required for food consumption (2003) and were classified as unsuitable for food consumption (U). All the other samples were classified as suitable for food consumption (S) (Table 3). In 2010 and 2011, 10.7 % and 8.7 %, respectively, of the grain samples did not meet the STS criteria (2003).

Table 2. Technological parameter values and grades for the wheat samples collected in 2010 and 2011.

Harvest	Analysed		M %	Wv g l ⁻¹	PC %	WG %	GI	FN s	ZS ml
2010	103	Mean	13.5	785.8	13.1	29.2	73.3	332.7	39.3
		Median	13.5	786.0	13.1	29.7	77.0	346.0	40.0
		Min.	11.8	713.0	10.3	18.6	15.0	185.0	21.0
		Max.	14.7	869.0	16.3	39.4	98.0	409.0	64.0
		Grade	E	E	E	E	-	E	E
2011	103	Mean	13.0	803.9	12.0	27.3	80.2	269.7	39.6
		Median	13.0	811.0	12.0	27.3	89.0	279.0	40.0
		Min.	11.2	698.0	8.9	12.8	8.0	91.0	16.0
		Max.	15.1	850.0	14.8	40.1	99.0	400.0	61.0
		Grade	E	E	A	E	-	E	E
Total	206	Mean	13.3	794.9	15.6	28.2	76.8	301.2	39.5
		Median	13.3	797.5	12.5	28.5	84.0	307.5	40.0
		F	34.27	19.84	40.63	8.45	6.26	60.71	0.07
		p	0.00*	0.00*	0.00*	0.00*	0.01*	0.00*	0.79

M – moisture, Wv – weight volume, PC – protein content, WG – wet gluten content, GI – gluten index, FN – falling number, ZS – Zeleny sedimentation index, E – elite quality, A – standard quality, F – value, p – value, * significant difference based on Tukey at p < 0.01

Table 3. The mean technological parameter values of samples that were suitable and unsuitable for human consumption according to the STS (2003).

Parameter	Harvest				Total	
	2010		2011		Total	
	S (94)	U (9)	S(92)	U (11)	S (186)	U (20)
M	13.50	13.54	12.98	13.39	13.24	13.46
Wv	789.68	745.44	808.61	764.91	799.04	756.15
PC	13.20	12.06	12.14	10.97	12.68	11.46
WG	29.58	24.72	28.02	21.19	28.81	22.78
GI	73.27	73.11	79.60	85.64	76.40	80.00
FN	332.88	330.22	280.02	183.73	306.74	249.65
ZS	39.91	33.22	40.46	32.73	40.18	32.95

(N) – Number, S – suitable for human consumption, U – unsuitable for human consumption, M – moisture, Wv – weight volume, PC – protein content, WG – wet gluten content, GI – gluten index, FN – falling number, ZS – Zeleny sedimentation index

DON content in wheat samples classified according to the STS (2003)

The number of samples that were positive for DON content in 2010 and 2011 are summarized in Table 4. The mean grain contamination by DON for all samples was significantly different between the 2 years ($p < 0.000$). On average, 80.1 % of the analysed samples was contaminated. The percentage contamination was higher in 2010 (93.2 %) than in 2011 (66.9 %). Differences between the mean sample contaminations show that the samples taken in 2010 were 3.3 times more contaminated than the samples taken in 2011 and the median value was also higher in 2010. Exactly 77.9 % of the S samples was contaminated by DON and the number of contaminated samples was 29.6 % higher in 2010 than in 2011. In the U group, all samples were positive for DON content and their total average contamination level was 68.9 % higher than in the S samples. The DON content in the U samples in 2010 and 2011 was four times higher and 1.9 times higher, respectively, than it was in the S samples. The higher DON content in 2010 compared to 2011 is probably related to precipitation, which was higher in May (168 mm) and June (127 mm) in 2010 than in 2011 (May = 45 mm; June = 84 mm). Furthermore, average temperatures were lower in 2010 (May = 15.2°C; June = 19.2°C) than in 2011 (May = 16.1°C; June = 19.9°C). These data were accessed from meteorological weather stations that were located near the collection sites in Slovakia and showed that the average precipitation was greater in 2010 (May and June) when wheat was at the flowering and ripening stages, respectively. These results correspond with previously published data where there was a positive relationship between rainfall and DON content in wheat. The previous data were obtained from samples taken from wheat cultivated between 2004 and 2006 in Slovakia (Šliková et al. 2008). Weather conditions during the flowering and ripening stages of wheat were critical for FHB development. A previous study showed that high temperatures in central Poland during July and August in 2009, accompanied by high rainfall in July were responsible for the high DON levels in wheat (Wiśniewska et al. 2014). Chandelier and Nimal (2011) found a strong correlation between mean annual DON content and number of days with mean relative humidity above 80 % over a period starting 7 days before the mean flowering date and ending on the 16th day of flowering. The wet weather before harvest seems to be particularly bad for cereal contamination of mycotoxins as well as of DON (Bernhoft et al. 2013).

A negative relationship was observed between some of the evaluated technological parameters (WV, GI and ZS) and DON content (Table 5). These results agree with numerous scientific studies on the effects of fusarium infection on the technological quality traits of wheat. Siuda et al. (2010) reported that fusariosis had an impact on the grain weight of infested grain and Dexter et al. (1996) also observed that grain weight decreased as the number of fusarium damaged grains in samples increased. It has been found that the fungal protease produced by *Fusarium culmorum* may damage storage proteins (Wang et al. 2005) and this can influence the GI value. Dexter et al. (1996) reported a decrease in glutenins in wheat infected by *F. graminearum*, but no qualitative differences in gliadins were observed. According to Prange et al. (2005), the gluten network was not attacked by *Fusarium* spp. and only small changes in the glutenin levels were observed. Many studies have shown that there is a reduction in ZS after wheat had been artificially infected with *Fusarium* (Gärtner et al. 2008, Papoušková et al. 2011). There was also a reduction in FN (Papoušková et al. 2011). Wang et al. (2005) found that there was negative correlation between *Fusarium* protein equivalent and the sedimentation value of wheat flour after *Fusarium culmorum* infection. According to Gärtner et al. (2008), this indicates that although the total amount of protein remains quite stable, the infection may alter its quality.

Table 4. The occurrence of deoxynivalenol in wheat samples according to years.

Sample	Harvest	No. of samples		DON mg kg ⁻¹		
		Analysed/ positives ¹	Average	Min.	Max.	Median
S	2010	94/87	0.76	0.20	2.94	0.49
	2011	92/58	0.27	0.20	2.12	0.20
	Total	186/145	0.52	0.20	2.94	0.20
U	2010	9/9	3.07	0.27	7.88	1.89
	2011	11/11	0.53	0.22	1.75	0.33
	Total	20/20	1.67	0.22	7.88	0.40
Total	2010	103/96	0.96*	0.20	7.88	0.51
	2011	103/69	0.29	0.20	2.12	0.20
	Total	206/165	0.63	0.20	7.88	0.25

S – suitable for human consumption, U – unsuitable for human consumption, DON – deoxynivalenol,

* significant difference based on Tukey test at $p < 0.01$

¹Positive samples: mycotoxin concentration above detection limit > 0.2 mg kg⁻¹,

Table 5. Correlations among the technological parameters and deoxynivalenol content.

Parameter	M	Wv	PC	WG	G I	FN	ZS
DON	0.097	- 0.316**	0.079	-0.008	-0.169*	0.072	-0.165*

M – moisture (%), Wv – weight volume (g l⁻¹), PC – protein content (%), WG – wet gluten content (%),

GI – gluten index, FN – falling number (s), ZS – Zeleny sedimentation index (ml),

DON – deoxynivalenol (mg kg⁻¹)

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level

DON occurrence in different grades of S wheat samples

Table 6 shows that the total occurrence of S samples contaminated by DON in grades E, A, B and P ranged from 58.1 % to 86.1 % and the lowest percentage occurrence of positive samples was found in the P grade (wheat suitable for bread-making). Differences between 2010 and 2011 in the occurrence of positive samples were the highest in grades E (33.3 %) and P (32.4 %). The total average contamination of the S samples was the highest for the B grade. In 2010, the mean contamination of the S sample quality grades ranged from 0.54 mg kg⁻¹ to 1.04 mg kg⁻¹. However, in 2011 the difference between the grades was only 0.05 mg kg⁻¹. The median levels were the same for both years in the P grade samples. However, they were higher in the other grades in 2010.

Excessive DON content in wheat samples

Samples with excessive DON content were found. The limit for DON content imposed by the European Union (1.25 mg kg⁻¹) was exceeded in 11.7 % (24 samples) of the samples analyzed (Table 6). The limit is the current maximum level allowed in the European Union for unprocessed cereals other than durum wheat, oats and maize (European Commission 2006). Samples with excessive DON content occur when favorable climatic conditions are created for the development of FHB disease at the same time as other factors that support the development of the disease are present, such as ongoing precipitation during harvest, an unsuitable preceding crop (mainly maize) or no fungicides were applied (Koch et al. 2006). Edwards (2009) states that the percentage of samples above these limits will fluctuate in the coming years.

Table 6. The occurrence of, and deoxynivalenol content in, wheat classified according to the STS (2003).

Harvest	G	Grade	DON mg kg ⁻¹				P ² - (%)
			Analysed/P ¹ - (%)	Average	Max.	Median	
2010	S	E	37/34 –(91.9)	0.74	2.94	0.50	4 – (10.8)
		A	28/27 – (96.4)	0.67	2.20	0.52	3 – (10.7)
		B	19/18 – (94.7)	1.04	2.80	0.58	6 – (31.5)
		P	10/8 – (80.0)	0.54	2.01	0.20	2 – (20.0)
	U	9/9 – (100.0)	3.07	7.88	1.89	5 – (55.5)	
2011	S	E	29/17 – (58.6)	0.29	2.12	0.20	1 – (3.4)
		A	25/18 – (72.0)	0.24	1.24	0.20	1 – (4.0)
		B	17/13 – (76.5)	0.25	0.61	0.20	0 – (0.0)
		P	21/10 –(47.6)	0.27	0.75	0.20	0 – (0.0)
	U	11/11 – (100.0)	0.53	1.75	0.33	2 – (18.2)	
Total	S	E	66 /51 – (72.3)	0.55	2.94	0.33	5 – (7.5)
		A	53/45 – (84.9)	0.47	2.20	0.20	4 – (7.5)
		B	36/31 – (86.1)	0.67	2.80	0.26	6 – (16.6)
		P	31/ 18 – (58.1)	0.36	2.01	0.20	2 – (6.5)
	U	20/20 – (100)	1.67	7.88	0.37	7 – (35.0)	

G – Group, ¹Positive samples – mycotoxin concentration above detection limit > 0.2 mg kg⁻¹, ²Positive samples – mycotoxin concentration above detection limit > 1.25 mg kg⁻¹, S – suitable for human consumption, U – unsuitable for human consumption, E – elite quality, A – standard quality, B – minimum quality requirements for intervention purchase of wheat, P – biscuits quality

The occurrence of samples with excessive DON content in Great Britain between 2001 and 2005 ranged from 0.4 % to 11.3 % (Edwards 2009). In Slovakia, between 2004 and 2008, the occurrence of excessive DON levels ranged from 0.0 % to 4.3 % in samples collected from the maize production region (Šliková et al. 2008). In this study, the excessive sample occurrence differed between the years. In 2011, the allowed limit was exceeded in 3.7 % of the samples and in 2010 it was 19.4 %. Table 6 shows that excessive samples occurred among the S and U samples and among the different S sample quality grades. We found out that wheat samples with good technological parameters can contain excessive amounts of DON. Our results were also supported by Polišíenská (2011), who found a wheat sample in the Czech Republic in 2010 that contained an excessive DON content (1.559 mg kg^{-1}), even though it had very good technological parameters ($Wv = 796 \text{ g l}^{-1}$, $FN = 345 \text{ s}$, $ZS = 52 \text{ ml}$ and $N = 13.5 \%$). Prange et al. (2005) came to the conclusion that high *Fusarium* infection levels, accompanied by high DON contents, did not necessarily reduce baking quality. According to our results, the highest percentage of samples exceeding the permitted DON limit occurred in the U samples and the highest DON levels were also found in these samples. In 2010, when samples were on average 3.3 times more contaminated than in 2011, half of the U samples contained excessive amounts of DON (Table 6).

Conclusion

These results show that there were significant differences in the technological quality of wheat grain and natural contamination by DON between 2010 and 2011. The occurrence and the level of DON contamination in samples suitable for human consumption were lower than in unsuitable samples. There were no differences in natural contamination of samples by DON among samples classified into grades according to their technological quality parameters. This research on the occurrence of the mycotoxin has also shown that samples with high technological qualities can contain dangerous amounts of DON. This inevitably implies that DON levels and incidence need to be controlled in wheat intended for human consumption and for feed.

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