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From wheat to sourdough bread: a laboratory scale study on the fate of deoxynivalenol content

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RESEARCH ARTICLE

Abstract

The effect of wheat processing by scouring, milling and sourdough bread making on the fate of deoxynivalenol was studied using wheat samples artificially contaminated with *Fusarium graminearum*. The scourer assures an intensive treatment of grain surface, allowing the reduction of deoxynivalenol content by 34.6-46.2%. After milling, the deoxynivalenol was found in all wheat mill fractions. The highest deoxynivalenol contents were found in bran fractions (bran and shorts). Concerning the flour fractions, those with low ash content, resulting from the first break and first reduction flours, were found to have higher deoxynivalenol concentrations than other flour fractions. The sourdough fermentation with selected lactic acid bacteria allowed the significant decrease (58.6-66.5%) of deoxynivalenol content of flour, while the spontaneous fermentation induced a much lower decrease (26.2-29.1%). The baking process caused the reduction of deoxynivalenol content by 11.4-15.5% compared to fermented dough.

Keywords: baking, bread, milling, mycotoxins, scouring, sourdough

1. Introduction

Fusarium spp. were frequently isolated from the wheat in different European countries such as France, Germany, Norway, Belgium, Poland, Italy, and the Netherlands (Chelkowski *et al.*, 2012; Edwards *et al.*, 2011; Fagnano *et al.*, 2012; Foroud and Eudes, 2009; Isebaert *et al.*, 2005; Krysinska-Traczyk *et al.*, 2007), therefore raising potential food security issues (Magan *et al.*, 2012). There are also some reports about damaging attacks in Romania, induced by *Fusarium* pathogens in wheat and maize, the most important staple food crops, cultivated on large areas (Ittu *et al.*, 2008; Tabuc *et al.*, 2009). Among *Fusarium* spp. mycotoxins, deoxynivalenol (DON) has been reported as being the most common mycotoxin contaminant associated with wheat and wheat-based products (Visconti and Pascale, 2010).

DON is a major metabolite produced by *Fusarium graminearum*. Commission Regulation (EC) no. 1881/2006 (EC, 2006) established the maximum DON level to 1,250 µg/kg for unprocessed wheat and 500 µg/kg for bread. In order to obtain safe end-products it is necessary to reduce wheat contamination by efficient cleaning, and technological processing such as milling and baking. In order to obtain final cereal products with mycotoxin contents within legislative limits, it is very important to understand the factors influencing the distribution of mycotoxins in the mill fractions (Edwards *et al.*, 2011).

Several studies showed that DON contamination could be reduced by separation of the shrivelled, broken, and small kernel at combi-cleaner together with indented separator or at density grader by impacting, scouring and aspiration (Abbas *et al.*, 1985; Eugster, 2002; Jouany, 2007; Kushiro, 2008; Tkachuk *et al.*, 1991). Moreover, the grains infected with *Fusarium* can have low-density and therefore can be removed through gravity separation (Kushiro, 2008) or by using an optical sorting system with visible camera and high-resolution infra-red sensors (Pearson *et al.*, 2004).

Milling has no direct effect on mycotoxin content in grains but during the milling process the mycotoxins are usually concentrated in certain mill fractions (Bullerman and Bianchini, 2007; Jouany, 2007); the highest content of mycotoxin was found in germs, bran and shorts. Most experiments on the effect of milling on mycotoxins distribution in mill fractions involved Buhler, MIAG and Allis Chalmers laboratory aggregates (Abbas *et al.*, 1985; Cheli *et al.*, 2010; Kushiro, 2008; Lancova *et al.*, 2009; Manasikan *et al.*, 2010; Seitz *et al.*, 1985). Cheli *et al.* (2010) studied the effect of conventional milling and debranning before milling on the distributions of contaminants (DON, cadmium and lead) in durum wheat mill fractions. They showed that debranning technology is more efficient in concentrating the contaminants in the animal feed products with respect to the conventional milling.

Visconti *et al.* (2010) studied DON distribution in durum wheat, semolina, and pasta and reported the highest levels of DON in the bran fraction, and lower levels in cleaned wheat and semolina (77 and 37%, respectively). The DON levels are even lower in spaghetti (33%), and in cooked spaghetti (20%), with respect to the uncleaned wheat (Visconti *et al.*, 2010).

In addition, the existence of conjugated form DON-3-glucoside (DON-3-Glc) has previously been reported in cereal products (De Angelis *et al.*, 2013; Kostelanska *et al.*, 2011; Suman *et al.*, 2013; Vaclavikova *et al.*, 2013). Suman *et al.* (2013) indicate the occurrence of DON and DON-3-Glc in bread, crackers, minicakes, and biscuits. In all tested samples the DON-3-Glc content was approximately 90% lower compared to the measured levels of DON in the same products.

Significant variations in DON-3-Glc content were observed when comparing minicakes/biscuits to crackers/bread, mainly as a consequence of the differences in terms of ingredients used, processing technologies and processing conditions.

DON is very stable during baking at temperatures ranging from 170 to 350 °C (Kushiro, 2008), but some recent reports indicate DON concentration changes during dough processing (Kostelanska *et al.*, 2011; Lancova *et al.*, 2009). Moreover Kostelanska *et al.* (2011) showed that DON-3-Glc content also decreases during baking.

Neira *et al.* (1997) and Boyacioglu *et al.* (1993) investigated the influence of adding yeasts and various ingredients to dough before baking, on DON levels. Kostelanska *et al.* (2011) showed that addition of enzymes mixtures caused the increase of conjugated DON-3-Glc in fermented dough up to 145%, followed by the decrease of both DON-3-Glc and DON (10 and 13%, respectively, compared to fermented dough) during baking. Concerning the effect of hydrolytic enzymes (α-amylase, cellulase, protease, and xylanase), Simsek *et al.* (2012) enlightened the increase in DON levels compared to the wheat composite. These results suggested that enzyme treatments cause DON binding or trapping to/into the cell wall matrix or protein component of the wheat kernel.

The objective of this study was to assess at the laboratory scale the effect of wheat processing by scouring, milling and sourdough bread making, on the fate of DON.

2. Materials and methods

Samples

The study was performed on Romanian wheat grains, Alex variety (harvest 2009), artificially contaminated with *F. graminearum* (MI 113 strain from USAMVB collection). Three wheat samples of 3 kg each, from the same lot, were spray-inoculated with *F. graminearum* spores (10^8-10^9 cfu/ml suspension, 80 ml/kg). The samples were stored for 3 months at 25 °C in sealed glass containers to ensure aw values of 0.97-0.98. At the end of the storage period the DON content was determined by the competitive enzyme immunoassay.

Scouring

Wheat samples were scoured using a laboratory scouring machine made by SC TEHNOPAN SA Bucharest (Pavel, 2006). The special design of the scouring machine allowed arranging the rotor tilting within certain degrees (30, 45 and 60 degrees) with respect to the horizontal axis. Abrasives grinding stones with granularity of 20, made of black carbide of silicon (SiC), with high content of silicon were used. After scouring, the dust particles from the products were removed using an in-house-made laboratory aspirator of dust particles.

Milling

After scouring the wheat samples were milled. The milling process was performed using an automatic laboratory mill (model MLU/202; Buhler, Uzwil, Switzerland). Three break flour fractions, three reduction flour fractions, and two bran fractions (bran and shorts) were obtained. The break and reduction flour fractions were mixed and further used for bread making.

Evaluation of physico-chemical properties

Moisture and ash contents were evaluated using the AACC 44-51 method (AACC, 2000) and the SR ISO 2171:2002 method (ASRO, 2008), respectively. pH measurements of the sourdough were made according to Romanian standard

methods 90/2007 (ASRO, 2008) by means of a Hanna digital pH-meter (Hanna Instruments, Kehl, Germany).

Bread preparation

Sourdoughs were prepared by well mixing in a large beaker the wheat flour with tap water and inoculum to get the dough yield (mass of dough/mass of flour ×100) of 300. After covering with aluminium foil, the beakers were placed in an incubator at 37 °C for 20 h. The inoculum used for preparing the wheat sourdough consisted of commercial lactic acid bacteria strains *Lactobacillus plantarum* and *Lactobacillus brevis* (DI-PROX MTTX, EDR Ingredients, Costișa, Romania), *L. plantarum* (EDR-P, EDR Ingredients) and *Lactobacillus helveticus* (LH-B02) (Chr Hansen, Hørsholm, Denmark). The inoculum was sized according the producer recommendations. The control samples were prepared with contaminated flours in the same conditions, without inoculum.

Dough formulations are detailed in Table 1. The dough was prepared at 28 °C by mixing all ingredients in a laboratory mixing device. After fermentation for 30 min at 28 °C in a laboratory proofer, the dough was divided in two pieces which were moulded and placed in baking trays. After a final leavening of 45 min, the trays were introduced into the oven. The samples were baked for 40 min at 260 °C (the steam tap was turned on 10-15 s before placing the samples into the oven).

Mycotoxin analysis

The mycotoxin contents of the wheat samples after scouring, milling fractions, sourdough, dough (after sourdough incorporation), proofed dough and bread were analysed. Dough and bread samples were first dried, ground, and sieved through a 60-mesh screen.

Mycotoxins were extracted from the ground samples with ultrapure water (0.055 μS/cm, using TKA MicroPure system, Niederelbert, Germany). The suspensions were first mixed vigorously for 3 min on a magnetic stirrer (Velp Scientifica, Usmate, Italy) and afterwards the extract was filtered.

Table 1. Formulations used to prepare the wheat bread samples with 20% sourdough.

	Amount
Wheat flour (g)	866
Sourdough (g)	400
Added water (ml)	284
Salt (g)	20
Compressed yeast (g)	15
Total water (ml)	550

The mycotoxin content was determined by the competitive enzyme immunoassay using the Ridascreen® DON test (R-Biopharm Rhone Ltd., Darmstadt, Germany) that was specially designed for quantitative analysis of DON in cereals, malt, feed, beer and wort. The concentration of DON was quantified according to the manufacturer's description. The optical density of the final extracts was measured at 450 nm using ELISA 96-well plate reader and the special software RIDA® Soft Win (R-Biopharm AG, Darmstadt, Germany) was afterwards used for mycotoxin content quantification. All sample solutions were analysed in duplicate. According to the manufacturer's description, the detection limit for DON by ELISA for cereals was 18.5 μg/kg. The mycotoxin content was expressed as μg per kg initial product.

Statistical analysis

The experiments were independently performed at least three times. The statistical significance of the data was analysed using the Student's t-test.

3. Results and discussion

At the end of three months storage period the levels of DON in the wheat samples were 271.1 μg/kg (sample A), 287.4 μg/kg (sample B) and 302.8 μg/kg (sample C). The contamination level is lower compared to the DON levels found in the south-eastern Romanian crop (Tabuc *et al.*, 2009).

Most contaminants of the grains are located on the kernel surface and it is possible to be partially removed by scouring (Eugster, 2002; Laca *et al.*, 2006). According to our results (Table 2), wheat scouring allowed reducing the DON content by 46.2, 41.7 and 34.6% for increasing levels of rotor tilting. The efficiency of mycotoxin content reduction was estimated by dividing the difference of the DON content before and after scouring to the initial content (Table 2). The scouring step also caused the wheat ash content decrease from 1.49% to 1.44%, 1.45% and 1.47%, when the rotor was set to 30, 45 and 60 degrees with respect to the horizontal position (Table 2).

The highest reduction of ash and DON contents were obtained when the rotor of the scourer was tilted at 30 degree with respect to the horizontal position. In this situation, the scourer accomplishes an intensive surface treatment of grain, and larger amounts of outer layers are removed. We obtained a higher efficiency of DON reduction compared to Nowicki *et al.* (1988), who reported the possibility of removing 22% of DON from grain surface by scouring.

Table 2. Deoxynivalenol (DON) content in wheat after scouring.

Sample code		Ash content (% dry weight)	DON content (µg/kg dry weight)	Percentage of DON reduction after scouring (%)
Wheat after scouring	A30	1.44	150.0	44.7
	B30	1.43	154.8	46.1
	C30	1.44	158.2	47.8
	mean±SD	1.44±0.01	154.3±4.10	46.2±1.56
	A45	1.46	164.6	39.3
	B45	1.45	165.8	42.3
	C45	1.45	171.5	43.4
	mean±SD	1.45±0.01	167.3±3.67	41.7±2.13
	A60	1.47	182.3	32.7
	B60	1.47	184.8	35.7
	C60	1.48	195.9	35.3
	mean±SD	1.47±0.01	187.7±7.24	34.6±1.61

A, B, C = sample name; 30, 45, 60 = rotor tilting from the horizontal position; SD = standard deviation.

The wheat samples with the lowest DON contents after scouring (A30, B30 and C30) were further used for the milling experiments. Weight proportions of the mill fractions are reported in Table 3.

The distributions of DON in the mill fractions obtained during the milling process of the three wheat samples are show in Table 4. After milling, the highest contents of DON were found in the bran fractions (bran and shorts). Considering the weight contribution of mill fractions to the total mass of wheat scouring taken for milling, most of the DON was contained in the bran and shorts, 31.5% and 22.9%, respectively. Lancova *et al.* (2009) reported that most of the DON was contained in the bran, 37-50%. The concentration of DON in bran and shorts is about 2.22 and 2.14-fold higher compared to the wheat after scouring, and 3.69 and 3.56-fold higher compared to the total flour. Similar results were reported by Gartner *et al.* (2008) and Trigo-Stockli *et al.* (1996) who used the same type of laboratory mill. Gartner *et al.* (2008) showed that bran had 5-fold higher DON content than the break flour, while the shorts had 3-fold higher DON content than the reduction flour. Trigo-Stockli *et al.* (1996) and Nishio *et al.* (2010) obtained flour and bran with about 50% and 100%,

Table 3. Weight proportions of the mill fractions (%).

Milling fractions	A30	B30	C30	mean±SD
Wheat scouring	100	100	100	100
First break flour (B1)	14.5	13.9	13.8	14.1±0.38
Second break flour (B2)	13.0	13.3	13.1	13.1±0.15
Third break flour (B3)	5.8	5.9	5.5	5.7±0.21
First reduction flour (R1)	24.0	23.1	24.0	23.7±0.52
Second reduction flour (R2)	15.6	15.1	15.4	15.4±0.25
Third reduction flour (R3)	3.2	3.2	3.1	3.2±0.06
Bran	13.9	14.5	14.1	14.2±0.31
Shorts	10.0	11.0	11.0	10.7±0.58
Total flour	76.1	74.5	74.9	75.2±0.83

A, B, C = sample name; 30 = rotor tilting from the horizontal position; SD = standard deviation; B1 = first break flour; B2 = second break flour; B3 = third break flour; R1 = first reduction flour; R2 = second reduction flour; R3 = third reduction flour.

Table 4. Deoxynivalenol (DON) content (µg/kg dry weight) and DON distribution[1] (%) in milling fractions.

Milling fractions	A30		B30		C30		mean±SD	
	DON content (µg/kg dry weight)	DON distribution (%)	DON content (µg/kg dry weight)	DON distribution (%)	DON content (µg/kg dry weight)	DON distribution (%)	DON content (µg/kg dry weight)	DON distribution (%)
Wheat scouring	150.0	100	154.8	100	158.2	100	154.3±2.29	100
First break flour	103.4	10.0	106.8	9.6	107.7	9.4	106.0±2.29	9.7±0.31
Second break flour	95.2	8.3	96.0	8.2	93.7	7.8	95.0±1.18	8.1±0.28
Third break flour	82.7	3.2	83.3	3.2	87.8	3.1	84.6±2.80	3.1±0.08
First reduction flour	98.0	15.7	98.8	14.7	100.0	15.2	98.9±1.02	15.2±0.47
Second reduction flour	74.2	7.7	74.8	7.3	83.7	8.1	77.5±5.31	7.7±0.43
Third reduction flour	68.2	1.5	68.5	1.4	81.0	1.6	72.6±7.30	1.5±0.09
Bran	342.3	31.7	338.8	31.7	347.7	31.0	342.9±4.47	31.5±0.43
Shorts	325.2	21.7	330.2	23.5	337.07	23.4	330.8±5.97	22.9±1.02

A, B, C = sample name; 30 = rotor tilting from the horizontal position; SD = standard deviation.
[1] The total amount of DON in the wheat scouring is considered 100%; the contributions were calculated based on the results reported in Tables 2 and 3.

respectively, higher concentration of DON with respect to the wheat.

Concerning the DON content of the flour fractions, we found that the fractions with low ash content, arising from the first break and first reduction flours, had higher DON concentrations (DON distributions of 9.7 and 15.2%, taking into account the weight contribution of these mill fractions to the total mass of wheat scouring) than other flour fractions. We would have expected to find higher DON contents in the flour fractions with high ash content (third break and reduction flours), but in these fractions the DON distribution was lower (3.1 and 1.5%). One can explained this situation by the fact that, by fracturing the kernel at the first break rolls, the fine particles from the pericarp layers are sifted and get into the first break flour. Moreover, the first break rolls provide particles that are processed to flour by the first reduction passage (Seitz *et al.*, 1985). Second and third break flour fractions, as well as second and third reduction flour fractions, have the highest mineral content because they contain more aleurone and subaleurone tissues. In Figure 1 one can see that the DON and the ash contents of flour fractions have an opposite trend of variation within the same type of milling passage.

The total flours made by mixing the break and reduction fractions obtained through milling the A30, B30 and C30 wheat samples were used for the baking experiments. The

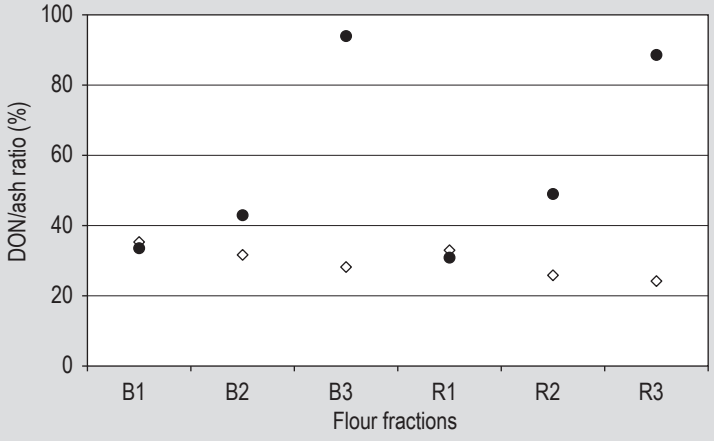

Figure 1. Ratio of deoxynivalenol (DON) in flour/scoured wheat (white diamonds) and ratio of ash in flour/scoured wheat (black circles) (B1 = first break flour; B2 = second break flour; B3 = third break flour; R1 = first reduction flour; R2 = second reduction flour; R3 = third reduction flour).

fate of the DON levels in different bread making stages, including the sourdough fermented with different types of lactic acid bacteria, is show in Table 5.

The pH of sourdoughs samples obtained by fermentation with the starter cultures DI-PROX MTTX, EDR-P and LH-B02 were 3.93, 3.97 and 3.84, respectively; much lower compared to the control sample (pH 5.44). The sourdough fermentation caused a significant decrease of the DON content (58.7-66.5%) with respect to the flour samples A30, B30 and C30. In case of spontaneous fermentation the decrease was much lower, of about 26.3-29.1%. Comparing the DON levels from the sourdough obtained with starter culture, one can see that the highest decrease was obtained in case of the sample fermented with DI-PROX MTTX (63.1-66.5%), and the lowest decrease was obtained in case of the sample fermented with LH-B02 (58.7-63.1%).

The DON levels of the dough fermented with yeast and 20% of sourdough with starter cultures decreased by 23.1-28.1% compared to the kneaded dough. In case of the control doughs prepared with spontaneously fermented sourdough, a decrease of 10.5-12.5% was obtained.

During bread baking for 40 min at 260 °C, the reduction of DON levels was about 11.4-15.5% compared to the fermented dough. Our results are in agreement with Kostelanska *et al.* (2011), who reported a decrease of DON during baking of 13%, compared to fermented dough.

In the recent years several studies highlighted the possibility of reducing the mycotoxin contamination of cereal products by fermentation (Dalie *et al.*, 2010; Halasz *et al.*, 2009). Garda *et al.* (2005) showed that partial mycotoxin decontamination is possible during alcoholic fermentation, while El-Nezami *et al.* (1998), Niderkon *et al.* (2006) and Gerez *et al.* (2009) confirmed that this is possible also using lactic acid bacteria. Our results in terms of DON fate during fermentation could be explained by the existence of DON-3-Glc in the artificially contaminated wheat samples. The low reduction of DON content after the spontaneous fermentation can be a consequence of the presence of DON-3-Glc, which cannot be detected by ELISA (Zachariasova *et al.*, 2008). Even if spontaneous microflora determined DON formation from DON-3-Glc during the fermentation step, it might have been metabolised by the yeasts, such as that DON concentration after fermentation is slightly lower compared to the flour. On the other hand DON content was not influenced when selected lactic acid bacteria were used for controlled fermentation; as suggested by Kostelanska *et al.* (2011), the bond between DON and glucose unit in DON-3-Glc may remain intact, instead the hydrolysis

Table 5. Evolution of the deoxynivalenol (DON) levels (µg/kg dry weight) during the bread making process, using different inocula (DI-PROX MTTX, EDR-P, LH-B02 or control).

Sample	DI-PROX MTTX	EDR-P	LH-B02	Control
A30				
Flour	91.1	91.1	91.1	91.1
Sourdough[1]	30.5 (66.5)	32.1 (64.8)	33.6 (63.1)	64.6 (29.1)
Kneaded dough	78.7	79.1	79.4	86.4
Fermented dough[2]	56.6 (28.1)	58.0 (26.7)	58.4 (26.4)	77.1 (10.8)
Bread[3]	47.9 (15.4)	49.0 (15.5)	49.6 (15.1)	67.2 (12.8)
B30				
Flour	92.2	92.2	92.2	92.2
Sourdough[1]	32.6 (64.5)	34.5 (62.6)	35.0 (62)	66.0 (28.4)
Kneaded dough	80.1	80.4	80.6	87.0
Fermented dough[2]	58.5 (27)	59.8 (25.6)	60.5 (24.9)	77.9 (10.5)
Bread[3]	50.4 (11.4)	51.1 (14.5)	51.8 (14.4)	67.5 (13.4)
C30				
Flour	95.1	95.1	95.1	95.1
Sourdough[1]	35.0 (63.2)	37.4 (60.7)	39.3 (58.7)	70.1 (26.3)
Kneaded dough	82.1	83,1	83.5	89.9
Fermented dough[2]	62.8 (23.5)	64.0 (23)	64.2 (23.1)	78.7 (12.5)
Bread[3]	53.4 (15)	54.1 (15.5)	55.8 (13.1)	67.9 (13.7)

[1] Decrease of DON content in sourdoughs compared to flours (%).

[2] Decrease of DON content in fermented dough compared to kneaded flour (%).

[3] Decrease of DON content in kneaded compared to bread (%).

of the R-glycosidic bonds between DON-3-Glc and cell polysaccharides might occur. The differences in terms of DON content within fermented dough samples prepared with lactic acid bacteria inoculum or by spontaneous fermentation, are not significant. Moreover no differences could be found within different types of bread samples. Kostelanska *et al.* (2011) showed that baking determine the reduction of both DON and DON-3-Glc contents.

4. Conclusions

The scourer assures an intensive surface treatment of grain allowing the removal of o large part of contaminants. After milling, the DON content was found in all wheat fractions. The highest contents of DON were found in the bran fractions (bran and shorts). The first break and first reduction flours were the most contaminated flour fractions.

The DON levels changed during the whole process of bread making. The most significant reduction of DON contents was obtained during sourdough fermentation, while during baking some decrease of DON, compared to fermented dough, was registered.

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Conflict of interest

The authors have no conflict of interest to declare.

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