HAL

archives-ouvertes.fr

Impact of durum wheat milling on the deoxynivalenol distribution in the outcoming fractions

Gisella Rios, Nadine Zakhia-Rozis, Marc Chaurand, Florence Richard-Forget, Marie-Françoise Samson, Joel Abecassis, Valérie Lullien-Pellerin

▶ **To cite this version:**

HAL Id: hal-00577326

https://hal.archives-ouvertes.fr/hal-00577326

Submitted on 17 Mar 2011

Impact of durum wheat milling on the deoxynivalenol distribution in the outcoming fractions

Journal:	*Food Additives and Contaminants*
Manuscript ID:	TFAC-2008-095.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	25-Jul-2008
Complete List of Authors:	RIOS, Gisella; INRA, UMR 1208 IATE ZAKHIA-ROZIS, Nadine; CIRAD, UMR 1208 IATE CHAURAND, MARC; INRA, UMR 1208 IATE RICHARD-FORGET, Florence; INRA, UR 1264 SAMSON, Marie-Françoise; INRA, UMR 1208 IATE ABECASSIS, JOEL; INRA, UMR 1208 IATE LULLIEN-PELLERIN, Valérie; INRA, UMR 1208 IATE
Methods/Techniques:	Chromatography - HPLC, Traceability
Additives/Contaminants:	Mycotoxins
Food Types:	Cereals and grain

SCHOLARONE™
Manuscripts

Impact of durum wheat milling on the deoxynivalenol distribution in the out-coming fractions

G. RÍOS[a], N. ZAKHIA-ROZIS[b], M. CHAURAND[a], F. RICHARD-FORGET[c], M.F. SAMSON[a], J. ABECASSIS[a] & V. LULLIEN-PELLERIN[a*].

[a]INRA, UMR 1208, Ingénierie des Agropolymères et Technologies Emergentes, 2 Place Pierre Viala, F-34060 Montpellier cedex1, France ; [b]CIRAD, UMR 1208, Ingénierie des Agropolymères et Technologies Emergentes, TA 40/16 73 Rue JF Breton 34398 Montpellier cedex 5, France ; [c]INRA, U.R. 1264, Mycologie et Sécurité des Aliments, Domaine de la Grande Ferrade, 71 Av. Edouard Bourleaux, BP 81, 33883 Villenave-d'Ornon cedex, France.

* Corresponding author. E-mail : lullien@supagro.inra.fr

1

Abstract

The milling behavior of two naturally infected samples of durum wheat grain with contrasting levels of mycotoxins was studied. Although the two samples showed a similar milling behavior, an increase of about twenty percent in deoxynivalenol (DON) levels was found in semolina from the sample containing the higher level of mycotoxin. However, even if the highest concentration of DON was found in fractions originating from the grain outer layers, the mycotoxin contamination in semolina and flours were not related to the amount of two compounds (ash or phytic acid) that could be used as markers to monitor these external tissues. The presence of the trichothecene-producing fungi in the most internal semolina fraction was also shown using specific DNA primers and PCR amplification. Comparison of DON concentrations in the feed stock and corresponding output at each milling step or grinding of semolina fractions followed by sizing showed that concentration of the mycotoxin occurs in the finest particles at the first processing steps. Therefore, DON contamination of the milling fractions is not simply due to the presence of peripheral grain tissues.

Key words: deoxynivalenol (DON), durum wheat, *Fusarium spp*., milling, mycotoxin, *Triticum*.

1 **Introduction**

2 *Fusarium* head blight (FHB) or scab is a worldwide disease of wheat *(Triticum aestivum* and

3 *durum)* and other small grain cereals which is caused by several *Fusarium* species (Parry et al.

4 1995; Bai et al. 2001). In Europe, *Fusarium graminearum* is one of the most frequently encountered

5 species on wheat (Goswami and Kistler 2004) mainly when wet and warm weather coincided with

6 the wheat anthesis and the beginning of grain filling (Bottalico and Perrone 2002; Champeil et al.

7 2004). This fungal disease leads to the formation of shrivelled, light test-weight kernels that reduce

8 grain yield and quality and affect milling yield (Edwards 2004).

9 In addition to grain yield loss due to FHB, production by *Fusarium* of toxic secondary metabolites

10 known as mycotoxins (Bennett and Klich 2003) may also impair the end-use of the harvested

11 cereals. One of these mycotoxins, deoxynivalenol (DON), which belongs to the trichothecene

12 (TCT) family could be considered as the most important wheat contaminant, as demonstrated by

13 surveys on its occurrence in grain (Bottalico and Perrone 2002). Its ingestion by animals was found

14 to lead to different symptoms depending on the sensitivity of species such as a decrease in weight

15 gain and nutritional efficiency, anorexic syndromes and altered immune function (Pestka 2007).

16 Due to its proved toxicity and its potential effect on the immune system, the European Community

17 have strictly fixed (EC regulation number 1881, 2006) the maximum DON authorized level in

18 unprocessed durum or common wheat grains at 1750 and 1250 $\mu g.kg^{-1}$ respectively and in resulting

19 flours or semolina at 750 $\mu g.kg^{-1}$.

20 Some control of DON contamination in the field could probably be obtained by the selection of

21 resistant cultivars (Gervais et al. 2003; Jiang et al. 2007) and a better knowledge of the

22 environmental factors which are involved (Edwards 2004; Koch et al. 2006). However, the

23 fractionation process, as one of the final steps leading to the cereal end-products, remains critical to

24 control the mycotoxin content in consumed fractions. If the first stages of *Fusarium* contamination

25 appear to be limited to the grain outer layers (McKeehen et al. 1999), contamination may be

1   reduced by the milling process that separates the endosperm from the bran containing these outer

2   layers, the fungi and the toxins could also be found in the endosperm (Kang and Buchenauer 1999;

3   Jackowiak et al. 2005) that leads to flours or semolina. Previous studies on durum wheat showed

4   that a significant reduction of the average DON level could be achieved at each step of the grain

5   processing (Nowicki et al. 1988; Dexter et al. 1997; Visconti et al. 2004; Manthey et al. 2004).

6   However, the relationship between these steps and the tissue origin of the different fractions have

7   not been investigated neither the effect of the level of contamination on the DON distribution.

8   In this paper, the grain characteristics and milling behavior at a semi-industrial scale of two

9   naturally infected samples of the same durum wheat cultivar but displaying distinctly different

10   levels of DON were analyzed. Furthermore, the impact of the milling process on the DON

11   distribution in the outcoming fractions was compared and discussed.

12

13   **Material and methods**

14   *Wheat samples and grain characterization*

15   Two samples of *Triticum durum* (*Acalou* cultivar) cultivated in France in 2003 were stored at 4°C

16   before milling.

17   Test weight and determination of Thousand Kernel Weight (TKW) determination were performed

18   respectively with standard AACC method 55-10 (AACC 2000) and ISO method (N°520, 1977).

19   Grain vitreousness was assessed by analysis of kernel cross-sections (obtained with a Pohl kernel

20   cutter, Versuchs und Lehranstalt für Brauerei, Berlin, Germany) and expressed by the percentage of

21   vitreous versus mealy grains.

22   Particle Size Index (PSI) was determined according to the adapted AACC method 55-30 (AACC

23   2000). Twenty grams of each wheat sample were ground with a laboratory mill 3303 (Perten

24   Instruments AB, Huddinge, Sweden) and the product obtained was sieved for 10 min using 75µm

25   sieve (LS-PRO, Hosokawa Alpine AG, Germany). The PSI corresponds to the percentage weight of

26   particles able to pass through the sieve.

1

Milling conditions

Cleaned grain was tempered to 17% water content before milling. Durum wheat milling was performed using a semi-industrial semolina mill (150 kg.h^{-1}) as described before (Chaurand et al. 1999). It was equipped with four break rolls or four reduction rolls followed by sifters for sizing and six sizing purifiers (Figure 1). This led to the production of four break flours (BF2 to BF5) and four reduction flours (RF1 to RF4); six purified semolina (SE1 and SE3 which corresponds to the inner endosperm, SE2 and SE4 originating from more external part of the endosperm compared with SE1 and SE3, SE5 and SE6 coming from the most peripheral part of the endosperm) and four bran or middlings: coarse bran (CB), purified fine bran (PFB), sized fine bran (SFB), and shorts (SH). Each fraction was collected and its yield expressed as weight percentage of the total milled grain amount. Furthermore, an aliquot sample of the 14 feeding fractions from each roll or purifier were also recovered. The collected fractions were stored at 4°C.

Figure 1

Biochemical analyses

Water and ash content of the grain were determined in triplicate according to standard AACC methods 44-19 and 08-12 respectively (AACC 2000). The phytic acid content was measured at 500 nm from acidic extract of ground grains or milling fractions using a colorimetric method described by Latta and Eskin (1980) and modified by Vaintraub and Lapteva (1988). A standard curve was obtained with corn phytate (P-8810, Sigma-Aldrich, St Quentin Fallavier, Fr.) solutions of known concentrations.

1 *DON measurement*

2   All the fractions were homogenized before their division to obtain a representative sample and

3 eventually ground in order to reduce the particle size below 500 μm. DON was then extracted at

4 room temperature with 100 mL of 84 % (v/v) acetonitrile in water using 25 g of each fractions

5 except for bran and middling fractions which allowed the amount to be reduced to 15 g. The

6 mixture was shaken 30 min and homogenized with an Ultra Turrax® for 3 min. After filtering

7 through Whatman® No. 1 filter paper, 8 mL of the filtrate were passed through a Mycosep® 225

8 column (Romer Labs., Union, MO, USA). The recovered purified solution (4 mL) was transferred

9 to a new vial and the solvent evaporated to dryness under a stream of nitrogen at 50°C. The dry

10 residue was then stored in a tightly closed glass bottle at −20°C until use or suspended in 500 μL of

11 methanol 20 % (v/v) and centrifuged (20000g, 6 min) before RP-HPLC analysis.

12   The DON extract (50 µL) was injected on a NovaPak® C_{18} column (60Å, 4 µm, 150 × 3.6 mm,

13 Waters SAS, Guyancourt, Fr.) and equilibrated with 7% (v/v) acetonitrile at a flow rate of 0.7

14 mL.min^{-1}. Elution was obtained by increasing the acetonitrile concentration in water to 80 % after

15 15 min. DON was detected at 218 nm with a 2487 dual λ UV detector (Waters SAS, Guyancourt,

16 Fr.). Commercial DON (Sigma-Aldrich, St Quentin Fallavier, Fr) was used to prepare calibration

17 standards ranging from 0.1 to 1 µg.mL^{-1}. To assess a calibration curve with the standards, a linear

18 regression was fitted on the data. DON concentration in the extracts were computed from the

19 regression curve. Mean DON recovery was estimated at 98.5 % with a 5.9 % coefficient of

20 variation from a wheat standard flour sample (FAPAS, T2210, with an average concentration of 463

21 µg.kg^{-1}) and based on triplicate measurement on four distinct extractions.

22

23 *Detection of TCT-producing Fusarium spp.*

24   DNA was extracted from ground grains (200 mg) or milling fractions with the DNeasy® Plant

25 Mini Kit from Qiagen (Qiagen S.A., Les Ulis, Fr.) according to the manufacturer instructions. All

26 PCR amplifications were carried out in 50 µl with 1ng of DNA template. Reactions mixtures

1   contained 25 pmoles of each primers (Tox5-1: 5'-GCTGCTCATCACTTTGCTCAG; Tox5-2: 5'-

2   CTGATCTGGTCACGCTCCATC) defined according to previous studies (Niessen and Vogel

3   1998), 2.5 U of DNA polymerase (Invitrogen Co., Cergy-Pontoise, Fr.), 400 µM of each

4   deoxynucleotide triphosphate (Promega, Charbonnières, Fr.). Reactions were performed using the

5   following PCR conditions: denaturation at 95°C for 1min 30, 40 cycles of denaturation at 95°C for

6   30 s, annealing at 55°C for 40 s and extension at 72°C for 30s, final extension at 72°C for 5 min,

7   followed by cooling at 4°C. Amplification products were analysed on a 1% agarose gel stained with

8   ethidium bromide. Genomic DNA extracted from the fungi *F. graminearum* was kindly given by L.

9   Pinson-Gadais (INRA, Bordeaux).

10

11   **Results and discussion**

12

13   *Wheat characterization and milling behavior*

14   Two durum wheat samples from the cultivar *Acalou* were selected as their levels of natural

15   contamination with DON were found to be different. The first sample (*A1*) was found to contain

16   382 µg.kg^{-1} (d. m.) of DON and thus could be considered acceptable in accordance to the European

17   Community legislation whereas the other (*A2*) displayed a DON level around 4203 µg.kg^{-1} (d. m.).

18   As *Fusarium* infection could lead to important grain damage that might severely affect the milling

19   behavior, the physical characteristics (test weight, TKW, vitreousness, PSI, water content) of wheat

20   grains from both samples were measured and compared.

21

22   **Table 1**

23

24   Table 1 clearly illustrates that even if the DON level in *A2* is more than ten fold higher than that in

25   *A1*, no apparent differences between grain characteristics of the two wheat grain samples were

26   observed. As these samples shared common grain characteristics, it was interesting to compare their

27   milling behavior, deoxynivalenol concentration and distribution in the generated fractions. The

1   yield of each fraction obtained after grain milling at a semi-industrial scale (150 kg.h^{-1}, milling

2   scheme in Figure 1) are summarized in Table 2. Even if the samples displayed a different level of

3   toxin, which could either be due to a different growth of the fungi or a distinct toxin production

4   level, no drastic changes were observed between the milling yields of the different fractions from

5   both of the samples, taking into account the coefficient of variation.

6

7   **Table 2**

8

9   *DON concentration and distribution in the milling fractions*

10   The DON concentration in all of the milling fractions were then determined and compared (Table

11   2). According to previous studies (Nowicki et al. 1988; Visconti et al. 2004), the most contaminated

12   fractions were the coarse bran (CB) and the purified fine bran (PFB). Indeed, these fractions contain

13   the major part of the grain outer layers that are the first to be infected by *Fusarium* (Jackowiak et al.

14   2005). These fractions showed a two to four fold concentration of DON compared to the grains as

15   demonstrated by the concentration ratio reported in Table 2.

16   Whatever the wheat sample, the other most contaminated fractions were the first two break flours

17   with DON concentration close to twice the toxin concentration in the grain. High amounts of DON

18   in these flours could be due to contamination with the outer layers at the grain breaking step or to

19   higher amounts of DON in the tissues from which these flours are originated. In contrast, the DON

20   concentration in the reduction flours however were found similar to those in the raw material. As

21   expected, the lowest concentration of DON was found in total semolina. However, the

22   concentration ratio between the toxin concentration in semolina and grains pointed out that

23   reduction of the DON concentration was more efficient for the less contaminated wheat sample (40

24   % in *A1* compared to 30 % in *A2*).

25   Comparison between distribution of the dry mass and DON amount in each type of the milling

26   fractions were calculated and summarized in Table 3.

1

2 **Table 3**

3   A distinct distribution of the total amount of DON in the milling fractions was obtained depending

4 on the wheat sample. Indeed, in the most contaminated grain sample, the highest toxin proportion

5 was found in semolina whereas, in the less contaminated sample, this highest toxin proportion was

6 found in bran and shorts. Two hypotheses could explain this difference in DON distribution: a

7 higher friability of the outer layers in the most contaminated grain sample or a higher amount of

8 toxin in the endosperm of these grains. In order to test a potential link between the increase of DON

9 proportion in semolina and contamination of this milling fraction with the outer layers, ash and

10 DON concentrations in semolina and flours were analyzed and compared (Figure 2).

11

12 **Figure 2**

13

14   Results clearly demonstrated that fractions displaying the highest DON concentration were not the

15 same as those showing the highest ash level. This lack of correlation between ash and DON

16 concentration was observed in both of the durum wheat samples. Therefore, it suggests that the

17 highest amount of DON in semolina from the *A2* sample could not be simply due to the presence of

18 grain outer layers in these fractions. These results are in accordance with a previously published

19 study dealing with common wheat milling fractions (Seitz et al. 1985). Furthermore, the phytic acid

20 concentration, that could be used to track the aleurone cell content (Greffeuille et al. 2005), was

21 also determined in semolina and flours from the most infected grains. Results confirmed the

22 absence of relationships between DON concentration in these milling fractions and presence of the

23 grain peripheral tissues. Indeed, the phytic acid concentration was found to increase from the inner

24 to the outer endosperm semolina (5.0 mg.g^{-1} d.m. in SE1 to 15.5 mg.g^{-1} d.m. in SE6) as well as

25 from the first break and reduction flours compared to the last one (respectively between 12.6 and

26 27.0 mg.g^{-1} d.m. from BF2 to BF5 and between 15.2 to 34.6 mg.g^{-1} d.m. from RF1 to RF4).

1 Therefore, the observed higher DON concentration in flours from the first break and reduction rolls

2 compared to the last ones was not only due to a contamination with the grain outer layers but rather

3 resulted from highly infected particles obtained at the first steps of grain breaking. Production of

4 these fine particles from infected parts of the endosperm could be generated by the fungus

5 penetration and by degradation of this tissue as already suggested for common wheat (Seitz et al.

6 1985)**.**

7

8 *Fusarium detection in the milling fractions*

9 In order to monitor the TCT-producing *Fusarium* species, DNA extraction was performed from

10 each type of milling fractions and amplified by PCR. The primers used for amplification were

11 derived from the gene *Tri5* encoding the first enzyme (trichodiene synthase) involved in the TCT

12 biosynthetic pathway, that was already been demonstrated to be a pertinent probe to track TCT-

13 producing fungi (Niessen and Vogel 1998). Results of the PCR amplification, reported in Figure 3,

14 revealed the expected specific fragment (658 bp).

15

16 **Figure 3**

17

18 All of the analyzed fractions were found to contain TCT-producing fungi. A higher amount of

19 fungi appeared in the fractions enriched in the grain peripheral tissues, such as coarse bran (CB) and

20 other bran and short fractions. But semolina coming out from the inner endosperm (SE1 and SE3)

21 was also found to contain TCT-producing fungi. These data are in accordance with previous results

22 obtained by Pinson-Gadais *et al.* (2007) however in a distinct durum wheat cultivar. Furthermore,

23 the fractions from the most contaminated grains also appeared to contain a higher amount of fungi.

24 Therefore, DON contamination in semolina could be due to the toxin production by fungi inside the

25 endosperm tissue.

26

1   *Relationship between roll and purifier feedings and corresponding outputs*

2   In order to clarify the DON distribution along milling, DON concentration in the feedings from all

3   rolls and purifiers were determined and summarized in Table 4. BR2 corresponds to meal fraction

4   obtained after the two first break rolls, BR3 to BR5 are the feedings corresponding to the following

5   break rolls, SP1 to SP6 are the feedings from the first to the sixth purifiers and RR1 to RR4 are the

6   feedings from the first to the fourth reduction rolls (Figure 1).

7

8   **Table 4**

9

10   The DON concentration in the feedings from each roll and purifier and the corresponding

11   obtained fraction (semolina or flours) could thus be compared: SP1 to SP6, BR2 to BR5 and RR1 to

12   RR4 with respectively SE1 to SE6, BF2 to BF5 and RF1 to RF4 as reported in Table 2. If DON

13   concentration in semolina was always found lower than those of the corresponding feedings

14   (ranging from identical to two or three-fold less concentrated), the ratio between break and

15   reduction flours and the corresponding feedings decreased from the first to the last step of the

16   milling process. Indeed, the DON concentrations were found to be 1.5 to 3-fold higher in the first

17   break and reduction flours compared to the corresponding feedings. Thus, at the first breaking and

18   reduction steps of the milling process, DON appeared to be concentrated in the flours, i.e. particles

19   with the smallest size.

20

21   **Table 5**

22

23   In order to test the hypothesis that grain breaking or endosperm reduction at the first milling steps

24   could lead to a preferential DON concentration in the finest fractions, the semolina SE3 and SE1

25   (originating from the most internal part of the grain) from sample *A2* were sifted. Comparison

1   between dry mass of the sifted semolina and their respective DON concentration showed that the

2   highest DON concentration occurred in the finest fractions (Table 5, a1, b1).

3   Each semolina was then ground, after removal of the finest particles (*i.e.* particles under 315 μm

4   for SE3 which accounted for 4 % of the semolina dry mass and displayed a DON concentration of

5   3358 μg.kg^{-1}; particles under 450 μm for SE1 which accounted for 26 % of the semolina dry mass

6   and displayed a DON concentration of 4151 μg.kg^{-1}). The resulting grinded material was classified

7   by size in order to analyze the DON concentration and distribution in each of the obtained fractions.

8   Table 5 (a2, b2) clearly shows that DON concentration was again higher (3 to 5 fold) in the fraction

9   characterized by the finest particle size.

10   Moreover, analysis of the DON amount distribution after semolina grinding and sizing compared

11   to those of the dry mass showed that DON amount in the largest size fractions was less than a half

12   the amount expected if DON was equally distributed in the material. In contrast, fractions

13   corresponding to the smallest particles concentrated the toxin with an about two fold factor

14   compared to the expected level.

15   Therefore, absence of relationships between DON contamination of flours and semolina and the

16   ash and phytic acid contents, as well as evidence of DON concentration in the finest particles from

17   the endosperm (Table 5) could explain the unexpected distribution of DON in the milling fractions.

18   It thus revealed that contamination of semolina or flours could not only be originated from the grain

19   outer layers but also from other contaminated parts of the grain that were shown to be friable. It

20   appeared particularly significant for grains containing a high level of mycotoxin and for fractions

21   coming out from the first steps of the process. Thus, higher DON level in the finest particles from

22   the semolina fraction of the most infected wheat sample could result from a deeper fungi

23   penetration in grains or a higher damage of the grain internal structure due to an increased synthesis

24   of hydrolytic enzymes, that are known to be produced by *Fusarium* (Jackowiak et al 2005).

25   However, further works are needed to test this hypothesis and examine if it is also observed for

26   other durum wheat cultivars. Nevertheless, these studies point out the interest to control the

1   generation and distribution of the finest particles during milling, in order to lower the DON amount

2   in semolina. This could be potentially achieved by improving the sizing efficiency of the sifters

3   after the two first break rolls or by increasing the efficiency of the first two sizing purifiers in order

4   to better eliminate the finest particles at the first milling steps even if this will results in a decrease

5   of the semolina yield. Indeed as semolina are the fractions used for pasta making, this control could

6   constitute a key factor in order to reduce the DON level in corresponding consumed wheat products.

7

14

15

1 **References**

2 AACC. 2000. Approved Methods. 10th ed. Minnesota (US): American Association of Cereal

3   Chemists.

4 Bai GH, Plattner R, Desjardins A, Kolb F. 2001. Resistance to *Fusarium* head blight and

5   deoxynivalenol accumulation in wheat. Plant Breeding. 120: 1-6.

6 Bennett JW, Klich M. T. 2003. Mycotoxins. Clin. Microbiol. Rev. 16: 497-516.

7 Bottalico A, Perrone G. 2002. Toxigenic *Fusarium* species and mycotoxins associated with head

8   blight in small-grain cereals in Europe. Eur. J. Plant Pathol. 108: 611-624.

9 Champeil A, Doré T, Fourbet JF. 2004. *Fusarium* head blight: epidemiological origin of the effects

10   of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in

11   wheat grains. Plant Sci. 166: 1389-1415.

12 Chaurand M, Lempereur I, Roulland TM, Autran JC, Abecassis J. 1999. Genetic and agronomic

13   effects on semolina milling value of durum wheat. Crop Sci. 39: 790-795.

14 Dexter JE, Marchylo BA, Clear RM, Clarke JM. 1997. Effect of *Fusarium* head blight on semolina

15   milling and pasta-making quality of durum wheat. Cereal Chem. 74: 519-525.

16 Edwards SG. 2004. Influence of agricultural practices on *Fusarium* infection of cereals and

17   subsequent contamination of grain by trichothecene mycotoxins. Toxicol. Lett. 153: 29-35.

18 Gervais L, Dedryver F, Morlais JY, Bodusseau V, Negre S, Bilous M, Groos C, Trottet M. 2003.

19   Mapping of quantitative trait loci for field resistance to *Fusarium* head blight in an European

20   winter wheat. Theor. Appl. Genet. 106: 961-970.

21 Goswami RS, Kistler HC. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol.

22   Plant Pathol. 5: 515-525.

23 Greffeuille V, Abecassis J, Bar Lhelgouach C, Lullien-Pellerin V. 2005. Differences in the aleurone

24   layer fate between hard and soft common wheats at grain miling. Cereal Chem. 82: 138-143.

25 Jackowiak H, Packa D, Wiwart M, Perkowski J. 2005. Scanning electron microscopy of *Fusarium*

26   damaged kernels of spring wheat. Int. J. of Food Microbiol. 98: 113-123.

1   Jiang G-L, Dong Y, Shi J, Ward RW. 2007. QTL analysis of resistance to *Fusarium* head blight in

2   the novel wheat germplasm CJ 9306. II. Resistance to deoxynivalenol accumulation and grain

3   yield loss. Theor. Appl Genet. 115: 1043-1052.

4   Kang Z, Buchenauer H. 1999. Immunocytochemical localization of fusarium toxins in infected

5   wheat spikes by *Fusarium culmorum*. Physiol. and Mol. Plant Pathol. 55: 275-288.

6   Koch HJ, Pringas C, Maerlaender B. 2006. Evaluation of environmental and management effects on

7   *Fusarium* head blight infection and deoxynivalenol concentration in the grain of winter wheat.

8   Europ. J. of Agron. 24: 357-366.

9   Latta M, Eskin M. 1980. A simple and rapid colorimetric method for phytate determination. J.

10   Agric. Food Chem. 28: 1313-1315.

11   Manthey FA, Wolf-Hall CE, Yalla S, Vijayakumar C, Carlson D. 2004. Microbial loads,

12   mycotoxins, and quality of durum wheat from the 2001 harvest of the Northern Plains region of

13   the United States. J. Food Prot. 67: 772-780.

14   McKeehen JD, Busch RB, Fulcher RG. 1999. Evaluation of wheat (*Triticum aestivum L.*) phenolic

15   acids during grain development and their contribution to *Fusarium* resistance. J. Agric. Food

16   Chem. 47: 1476-1482.

17   Niessen ML, Vogel RF. 1998. Group specific PCR-detection of potential trichothecene producing

18   *Fusarium* species in pure cultures and cereal samples. Syst. Appl. Microbiol. 21: 618 - 31.

19   Nowicki TW, Gaba DG, Dexter JE, Matsuo RR, Clear RM. 1988. Retention of the *Fusarium*

20   mycotoxin deoxynivalenol in wheat during processing and cooking of spaghetti and noodles. J.

21   Cereal Sci. 8: 189–202.

22   Parry DW, Jenkinson P, McLeod L. 1995. *Fusarium* ear blight (scab) in small grain cereals, a

23   review. Plant Pathol. 44: 207-238.

24   Pestka JJ. 2007. Deoxynivalenol: Toxicity, mechanisms and animal health risks. Anim. Feed Sci.

25   and Technol. 137: 283-298.

26   Pinson-Gadais L, Barreau C, Chaurand M, Gregoire S, Monmarson M, Richard-Forget F. 2007.

1 Distribution of toxinogenic *Fusarium spp*. and mycotoxin production in milling fractions of

2 durum wheat. Food Additiv. and Contam. 24: 53-62.

3 Seitz LM, Yamazaki WT, Clements RL, Mohr HE, Andrews L. 1985. Distribution of

4 deoxynivalenol in soft wheat mill streams. Cereal Chem. 62: 467-469.

5 Vaintraub IA, Lapteva NA. 1988. Colorimetric determination of phytate in unpurified extracts of

6 seeds and the products of their processing. Anal. Biochem. 175: 227-230.

7 Visconti A, Haidukowski EM, Pascale M, Silvestris M. 2004. Reduction of deoxynivalenol during

8 durum wheat processing and spaghetti cooking. Toxicol. Lett. 153: 181-189.

9

10

11

12

13

14

15

16

17

18

19

20

21
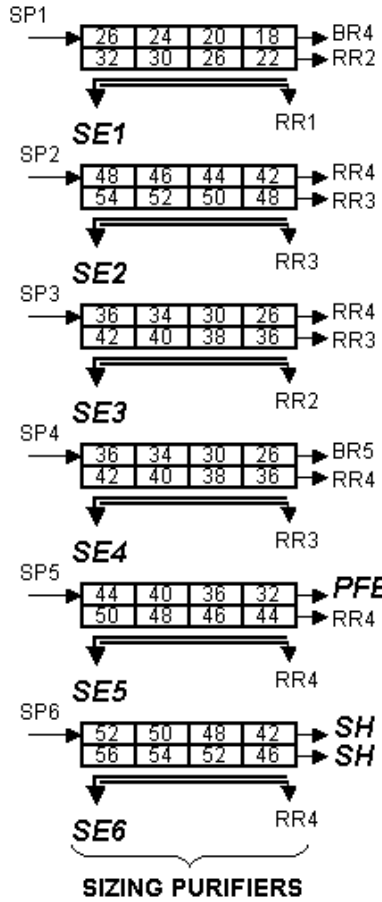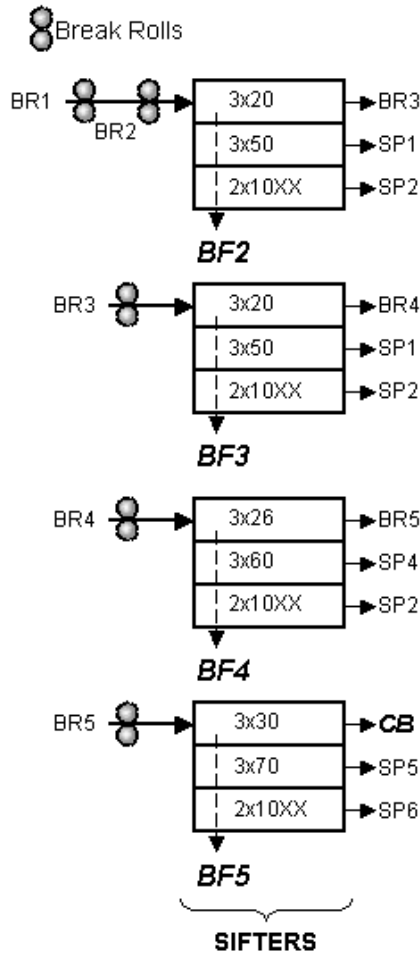
22

Figure captions

Figure 1: Milling diagram of the semi industrial pilot mill (INRA, Montpellier). BR1 to BR5 are feedings of break rolls, BF2 to BF5 are corresponding break flours, SP1 to SP6 are feedings of purifiers, SE1 to SE6 are semolina; RR1 to RR4 are feedings of reduction rolls, RF1 to RF4 are reduction flours, CB, PFB and SFB are respectively coarse bran, purified fine bran and sized fine bran, SH are shorts.

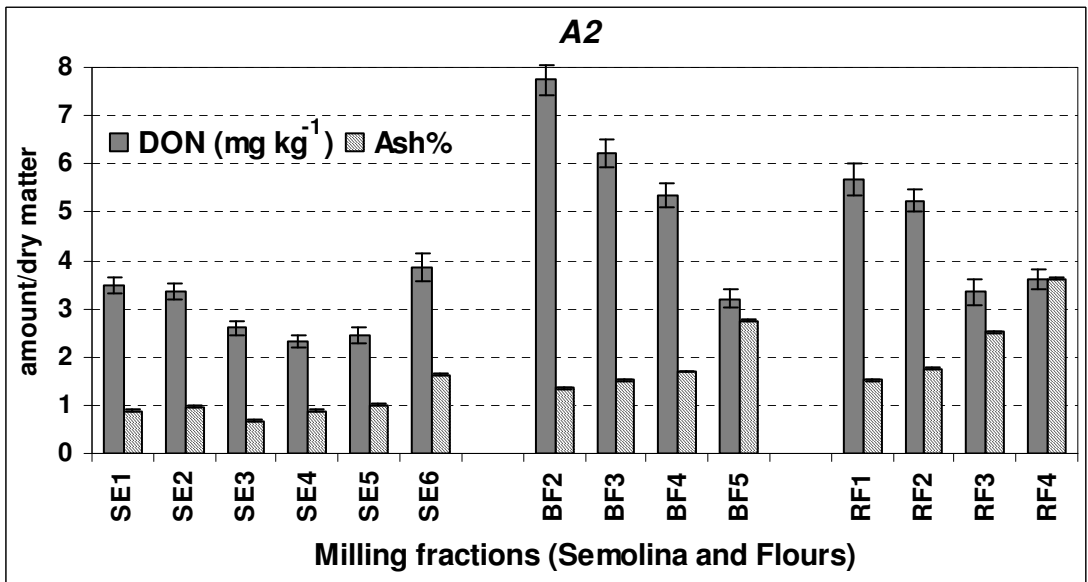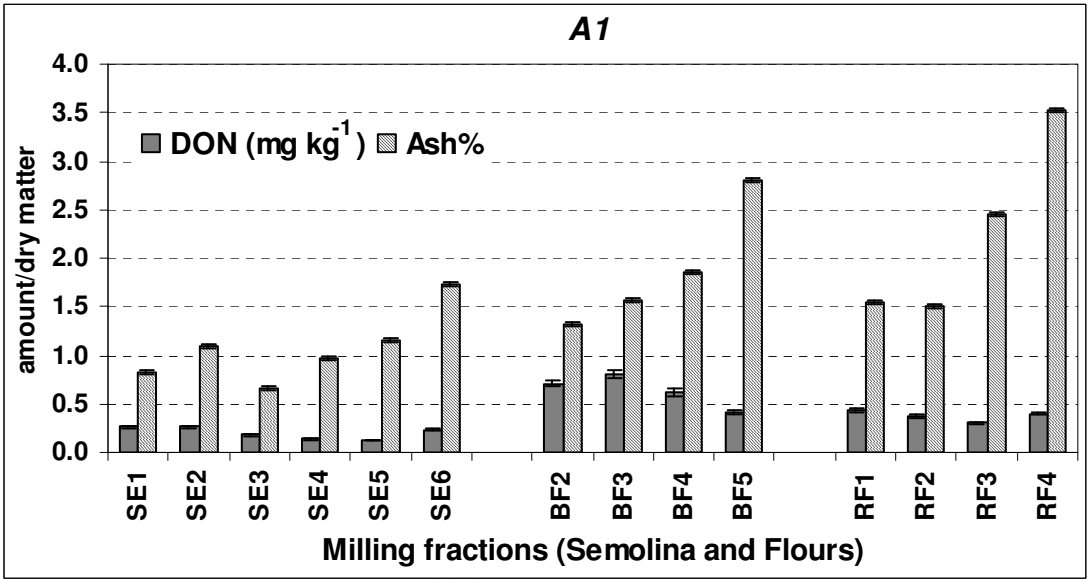**Figure 2**: Comparison of DON (mg.kg^{-1} d. m.) and ash (% d. m.) concentration in the milling fractions (except brans and shorts) from the two durum wheat samples (means of two replicates).

Figure 3 : Detection of TCT-producing fungi in grains and milling fractions from durum wheat *Acalou* samples *A1* and *A2* after PCR with primers derived from the gene encoding trichodiene synthase. *F. graminearum* corresponds to DNA extracted from the fungus. ST are total semolina, SE1 and SE3 are semolina from the most inner part of the endosperm, BF2 are the first break flours, RF1 are the first reduction flours, CB, SH, SFB and PFB are respectively coarse bran, shorts, sized fine bran and purified fine bran.

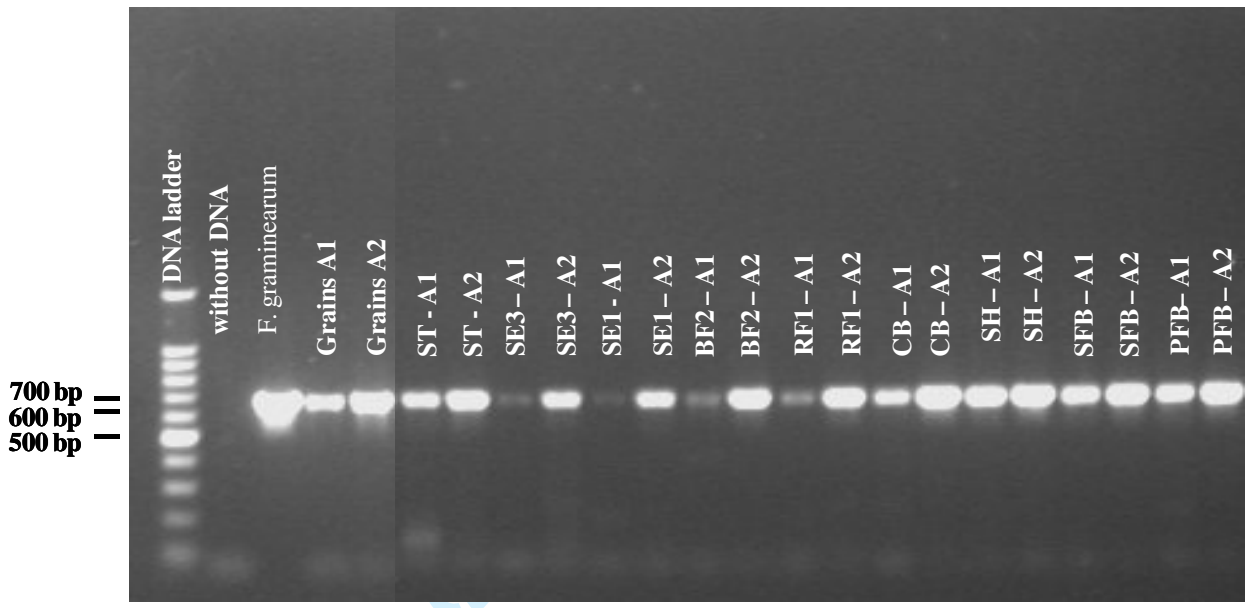Table 1: Main physical characteristics and total DON concentration of the analyzed *Acalou* wheat samples naturally infected with *Fusarium* [a].

Samples	*A1*	*A2*
DON (μg.kg^{-1} d. m.)	382.0 ± 9.7	4203.5 ± 202.7
Test weight (kg.hL^{-1})	84.5 ± 0.1	84.3 ± 0.1
TKW (g)	52.0 ± 0.6	50.0 ± 0.7
Vitreousness (%)	95.3	95.3
PSI (%)	5.1 ± 0.1	5.3 ± 0.1
Water content (%)	12.16 ± 0.01	12.33 ± 0.02

[a] Values are means and standard deviation of three replicates.

Table 2: Yield and DON concentration in the different milling fractions from the two durum wheat samples**.**

Milling Fractions		*A1* Yield[a] (% d. m.)	Concentrations DON[b] (µg.kg⁻¹ d.m⁻)	Ratio[c]	*A2* Yield[a] (% d. m.)	Concentrations DON[b] (µg.kg⁻¹ d.m⁻)	Ratio[c]
Semolina	SE1	6.2	266	0.70	6.0	3497	0.83
	SE2	26.0	260	0.68	25.3	3359	0.80
	SE3	26.8	180	0.47	25.9	2600	0.62
	SE4	10.5	139	0.36	10.5	2324	0.55
	SE5	0.1	121	0.32	0.7	2440	0.58
	SE6	6.8	230	0.60	7.5	3850	0.92
Total semolina		*76.4*	*220*	*0.58*	*75.9*	*2940*	*0.70*
Break flours	BF2	0.7	707	1.85	0.4	7737	1.84
	BF3	0.5	808	2.12	0.8	6206	1.48
	BF4	1.0	618	1.62	1.0	5341	1.27
	BF5	1.0	416	1.09	1.0	3208	0.76
Reduction flours	RF1	1.1	431	1.13	1.1	5679	1.35
	RF2	1.1	368	0.96	1.1	5239	1.25
	RF3	0.5	300	0.79	0.6	3349	0.80
	RF4	1.0	400	1.05	1.4	3601	0.86
Bran and Shorts	SFB	0.7	575	1.51	0.7	5196	1.24
	PFB	6.9	1146	3.00	7.2	10645	2.53
	CB	7.7	1463	3.83	7.6	14416	3.43
	SH	1.6	450	1.18	1.2	6091	1.45

[a] *C. V. < 5 %.*
[b] means of two replicates, *C. V. < 5%* for semolina and flours and < 10% for brans and shorts.
[c] Ratio between DON concentrations in the analyzed fraction versus those in cleaned grains.

Table 3: Comparison of yield and DON distribution in percent of the total grain mass or DON total content.

Milling Fractions	A1		A2	
	Yield[a] (% d.m.)	DON[b] (% d.m.)	Yield[a] (% d.m.)	DON[b] (% d.m.)
Total Semolina	76.4	40.8	75.9	49.6
Total Break Flours	3.2	4.8	3.3	3.7
Total Reduction Flours	3.7	3.5	4.2	4.1
Total Brans and Shorts	16.8	50.8	16.6	42.6

[a] Total yield was obtained from Table 2.
[b] calculated taking into account the fraction yield and DON concentration and expressed as a percentage of the total amount of DON in the sample.

Table 4: DON concentration (µg.kg^{-1} d.m.) in the feedings of rolls and purifiers and ratio between DON concentrations in flours or semolina (reported in Table 2) versus those in the feedings.

Fractions		A1		A2	
		DON[a]	Ratio	DON[a]	Ratio
Break roll feedings	**BR2**	250	2.83	4891	1.58
	BR3	369	2.19	5865	1.06
	BR4	300	2.06	5946	0.90
	BR5	594	0.70	8571	0.37
Sizing purifier feedings	**SP1**	262	1.01	4476	0.78
	SP2	352	0.74	4343	0.77
	SP3	200	0.90	3563	0.73
	SP4	201	0.69	3477	0.67
	SP5	320	0.38	5747	0.42
	SP6	349	0.66	4143	0.93
Reduction roll feedings	**RR1**	242	1.78	2719	2.09
	RR2	248	1.48	3450	1.52
	RR3	401	0.75	3561	0.94
	RR4	312	1.28	4640	0.78

[a] means of two replicates, *C. V.* < 7%.

Table 5: Distribution in dry mass (% d.m.), DON concentration (µg.kg^{-1} d.m.) and DON distribution (%) in sieved fractions from semolina SE3 or SE1 of the wheat sample *A2* before (a1, b1) and after (a2, b2) grinding. DON concentration of each analyzed fraction was specified in brackets at the top of each table.

a1

Sieve mesh size (mm)	Dry mass (% d.m.)	DON (µg.kg^{-1} d.m.)	DON (%)
SE3 A2 (2600 µg.kg^{-1} d.m.)			
> 0.560	8	2089	7
0.560- 0.450	31	2285	28
< 0.450	61	2716	66

a2

Sieve mesh size (mm)	Dry mass (% d.m.)	DON (µg.kg^{-1} d.m.)	DON (%)
SE3 A2 (2300 µg.kg^{-1} d.m.)			
> 0.200	26	967	11
0.100 - 0.200	28	1555	19
< 0.100	43	3682	69

b1

Sieve mesh size (mm)	Dry mass (% d.m.)	DON (µg.kg^{-1} d.m.)	DON (%)
SE1 A2 (3496 µg.kg^{-1} d.m.)			
> 0.630	24	2418	18
0.630 – 0.560	32	2956	29
< 0.560	44	3912	53

b2

Sieve mesh size (mm)	Dry mass (% d.m.)	DON (µg.kg^{-1} d.m.)	DON (%)
SE1 A2 (3189 µg.kg^{-1} d.m.)			
> 0.315	31	1087	11
0.160 - 0.315	30	1863	18
< 0.160	39	5736	71