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

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2 1 **Impact of durum wheat milling on the deoxynivalenol distribution in the out-**
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5 2 **coming fractions**
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3
4 2 **Abstract**
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7 3 The milling behavior of two naturally infected samples of durum wheat grain with contrasting levels of
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9 4 mycotoxins was studied. Although the two samples showed a similar milling behavior, an increase of about
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11 5 twenty percent in deoxynivalenol (DON) levels was found in semolina from the sample containing the higher
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13 6 level of mycotoxin. However, even if the highest concentration of DON was found in fractions originating
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15 7 from the grain outer layers, the mycotoxin contamination in semolina and flours were not related to the
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17 8 amount of two compounds (ash or phytic acid) that could be used as markers to monitor these external
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19 9 tissues. The presence of the trichothecene-producing fungi in the most internal semolina fraction was also
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21 10 shown using specific DNA primers and PCR amplification. Comparison of DON concentrations in the feed
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23 11 stock and corresponding output at each milling step or grinding of semolina fractions followed by sizing
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25 12 showed that concentration of the mycotoxin occurs in the finest particles at the first processing steps.
26
27 13 Therefore, DON contamination of the milling fractions is not simply due to the presence of peripheral grain
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29 14 tissues.
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35 16 **Key words:** deoxynivalenol (DON), durum wheat, *Fusarium spp.*, milling, mycotoxin, *Triticum*.
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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 $\mu\text{g.kg}^{-1}$ respectively and in resulting
19 flours or semolina at 750 $\mu\text{g.kg}^{-1}$.

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.

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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® C₁₈ 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 $\mu\text{g}\cdot\text{mL}^{-1}$. 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 $\mu\text{g}\cdot\text{kg}^{-1}$) and based on triplicate measurement on four distinct extractions.

22 *Detection of TCT-producing Fusarium spp.*

23 DNA was extracted from ground grains (200 mg) or milling fractions with the DNeasy® Plant
24 Mini Kit from Qiagen (Qiagen S.A., Les Ulis, Fr.) according to the manufacturer instructions. All
25 PCR amplifications were carried out in 50 μl with 1ng of DNA template. Reactions mixtures
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2 1 contained 25 pmoles of each primers (Tox5-1: 5'-GCTGCTCATCACTTTGCTCAG; Tox5-2: 5'-
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4 2 CTGATCTGGTCACGCTCCATC) defined according to previous studies (Niessen and Vogel
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6 3 1998), 2.5 U of DNA polymerase (Invitrogen Co., Cergy-Pontoise, Fr.), 400 μ M of each
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8 4 deoxynucleotide triphosphate (Promega, Charbonnières, Fr.). Reactions were performed using the
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10 5 following PCR conditions: denaturation at 95°C for 1min 30, 40 cycles of denaturation at 95°C for
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12 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,
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14 7 followed by cooling at 4°C. Amplification products were analysed on a 1% agarose gel stained with
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16 8 ethidium bromide. Genomic DNA extracted from the fungi *F. graminearum* was kindly given by L.
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18 9 Pinson-Gadais (INRA, Bordeaux).
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26 11 **Results and discussion**

28 12 *Wheat characterization and milling behavior*

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31 14 Two durum wheat samples from the cultivar *Acalou* were selected as their levels of natural
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33 15 contamination with DON were found to be different. The first sample (*A1*) was found to contain
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35 16 382 μ g.kg⁻¹ (d. m.) of DON and thus could be considered acceptable in accordance to the European
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37 17 Community legislation whereas the other (*A2*) displayed a DON level around 4203 μ g.kg⁻¹ (d. m.).
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39 18 As *Fusarium* infection could lead to important grain damage that might severely affect the milling
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41 19 behavior, the physical characteristics (test weight, TKW, vitreousness, PSI, water content) of wheat
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43 20 grains from both samples were measured and compared.
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51 22 **Table 1**

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55 24 Table 1 clearly illustrates that even if the DON level in *A2* is more than ten fold higher than that in
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57 25 *A1*, no apparent differences between grain characteristics of the two wheat grain samples were
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59 26 observed. As these samples shared common grain characteristics, it was interesting to compare their
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27 milling behavior, deoxynivalenol concentration and distribution in the generated fractions. The

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2 1 yield of each fraction obtained after grain milling at a semi-industrial scale (150 kg.h⁻¹, milling
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4 2 scheme in Figure 1) are summarized in Table 2. Even if the samples displayed a different level of
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6 3 toxin, which could either be due to a different growth of the fungi or a distinct toxin production
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8 4 level, no drastic changes were observed between the milling yields of the different fractions from
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10 5 both of the samples, taking into account the coefficient of variation.
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16 7 **Table 2**

17 8 18 19 20 9 ***DON concentration and distribution in the milling fractions***

21 10 The DON concentration in all of the milling fractions were then determined and compared (Table
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23 11 2). According to previous studies (Nowicki et al. 1988; Visconti et al. 2004), the most contaminated
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25 12 fractions were the coarse bran (CB) and the purified fine bran (PFB). Indeed, these fractions contain
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27 13 the major part of the grain outer layers that are the first to be infected by *Fusarium* (Jackowiak et al.
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29 14 2005). These fractions showed a two to four fold concentration of DON compared to the grains as
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31 15 demonstrated by the concentration ratio reported in Table 2.
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37 16 Whatever the wheat sample, the other most contaminated fractions were the first two break flours
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39 17 with DON concentration close to twice the toxin concentration in the grain. High amounts of DON
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41 18 in these flours could be due to contamination with the outer layers at the grain breaking step or to
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43 19 higher amounts of DON in the tissues from which these flours are originated. In contrast, the DON
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45 20 concentration in the reduction flours however were found similar to those in the raw material. As
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47 21 expected, the lowest concentration of DON was found in total semolina. However, the
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49 22 concentration ratio between the toxin concentration in semolina and grains pointed out that
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51 23 reduction of the DON concentration was more efficient for the less contaminated wheat sample (40
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53 24 % in A1 compared to 30 % in A2).
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59 25 Comparison between distribution of the dry mass and DON amount in each type of the milling
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26 fractions were calculated and summarized in Table 3.

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60**Table 3**

A distinct distribution of the total amount of DON in the milling fractions was obtained depending on the wheat sample. Indeed, in the most contaminated grain sample, the highest toxin proportion was found in semolina whereas, in the less contaminated sample, this highest toxin proportion was found in bran and shorts. Two hypotheses could explain this difference in DON distribution: a higher friability of the outer layers in the most contaminated grain sample or a higher amount of toxin in the endosperm of these grains. In order to test a potential link between the increase of DON proportion in semolina and contamination of this milling fraction with the outer layers, ash and DON concentrations in semolina and flours were analyzed and compared (Figure 2).

Figure 2

Results clearly demonstrated that fractions displaying the highest DON concentration were not the same as those showing the highest ash level. This lack of correlation between ash and DON concentration was observed in both of the durum wheat samples. Therefore, it suggests that the highest amount of DON in semolina from the A2 sample could not be simply due to the presence of grain outer layers in these fractions. These results are in accordance with a previously published study dealing with common wheat milling fractions (Seitz et al. 1985). Furthermore, the phytic acid concentration, that could be used to track the aleurone cell content (Greffeuille et al. 2005), was also determined in semolina and flours from the most infected grains. Results confirmed the absence of relationships between DON concentration in these milling fractions and presence of the grain peripheral tissues. Indeed, the phytic acid concentration was found to increase from the inner 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 from the first break and reduction flours compared to the last one (respectively between 12.6 and 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).

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2 1 Therefore, the observed higher DON concentration in flours from the first break and reduction rolls
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4 2 compared to the last ones was not only due to a contamination with the grain outer layers but rather
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6 3 resulted from highly infected particles obtained at the first steps of grain breaking. Production of
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8 4 these fine particles from infected parts of the endosperm could be generated by the fungus
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10 5 penetration and by degradation of this tissue as already suggested for common wheat (Seitz et al.
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12 6 1985).
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18 8 *Fusarium detection in the milling fractions*

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21 9 In order to monitor the TCT-producing *Fusarium* species, DNA extraction was performed from
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23 10 each type of milling fractions and amplified by PCR. The primers used for amplification were
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25 11 derived from the gene *Tri5* encoding the first enzyme (trichodiene synthase) involved in the TCT
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27 12 biosynthetic pathway, that was already been demonstrated to be a pertinent probe to track TCT-
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29 13 producing fungi (Niessen and Vogel 1998). Results of the PCR amplification, reported in Figure 3,
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31 14 revealed the expected specific fragment (658 bp).
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38 16 **Figure 3**

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42 18 All of the analyzed fractions were found to contain TCT-producing fungi. A higher amount of
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44 19 fungi appeared in the fractions enriched in the grain peripheral tissues, such as coarse bran (CB) and
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46 20 other bran and short fractions. But semolina coming out from the inner endosperm (SE1 and SE3)
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48 21 was also found to contain TCT-producing fungi. These data are in accordance with previous results
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50 22 obtained by Pinson-Gadais *et al.* (2007) however in a distinct durum wheat cultivar. Furthermore,
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52 23 the fractions from the most contaminated grains also appeared to contain a higher amount of fungi.
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54 24 Therefore, DON contamination in semolina could be due to the toxin production by fungi inside the
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56 25 endosperm tissue.
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2 1 *Relationship between roll and purifier feedings and corresponding outputs*

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4 2 In order to clarify the DON distribution along milling, DON concentration in the feedings from all
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6 3 rolls and purifiers were determined and summarized in Table 4. BR2 corresponds to meal fraction
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8 4 obtained after the two first break rolls, BR3 to BR5 are the feedings corresponding to the following
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10 5 break rolls, SP1 to SP6 are the feedings from the first to the sixth purifiers and RR1 to RR4 are the
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12 6 feedings from the first to the fourth reduction rolls (Figure 1).
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23 8 **Table 4**

24 10 The DON concentration in the feedings from each roll and purifier and the corresponding
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26 11 obtained fraction (semolina or flours) could thus be compared: SP1 to SP6, BR2 to BR5 and RR1 to
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28 12 RR4 with respectively SE1 to SE6, BF2 to BF5 and RF1 to RF4 as reported in Table 2. If DON
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30 13 concentration in semolina was always found lower than those of the corresponding feedings
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32 14 (ranging from identical to two or three-fold less concentrated), the ratio between break and
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34 15 reduction flours and the corresponding feedings decreased from the first to the last step of the
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36 16 milling process. Indeed, the DON concentrations were found to be 1.5 to 3-fold higher in the first
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38 17 break and reduction flours compared to the corresponding feedings. Thus, at the first breaking and
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40 18 reduction steps of the milling process, DON appeared to be concentrated in the flours, i.e. particles
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42 19 with the smallest size.
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54 21 **Table 5**

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56 23 In order to test the hypothesis that grain breaking or endosperm reduction at the first milling steps
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58 24 could lead to a preferential DON concentration in the finest fractions, the semolina SE3 and SE1
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60 25 (originating from the most internal part of the grain) from sample A2 were sifted. Comparison

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2 1 between dry mass of the sifted semolina and their respective DON concentration showed that the
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4 2 highest DON concentration occurred in the finest fractions (Table 5, a1, b1).

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6 3 Each semolina was then ground, after removal of the finest particles (*i.e.* particles under 315 μm
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8 4 for SE3 which accounted for 4 % of the semolina dry mass and displayed a DON concentration of
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10 5 3358 $\mu\text{g.kg}^{-1}$; particles under 450 μm for SE1 which accounted for 26 % of the semolina dry mass
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12 6 and displayed a DON concentration of 4151 $\mu\text{g.kg}^{-1}$). The resulting grinded material was classified
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14 7 by size in order to analyze the DON concentration and distribution in each of the obtained fractions.
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16 8 Table 5 (a2, b2) clearly shows that DON concentration was again higher (3 to 5 fold) in the fraction
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18 9 characterized by the finest particle size.

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24 10 Moreover, analysis of the DON amount distribution after semolina grinding and sizing compared
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26 11 to those of the dry mass showed that DON amount in the largest size fractions was less than a half
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28 12 the amount expected if DON was equally distributed in the material. In contrast, fractions
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30 13 corresponding to the smallest particles concentrated the toxin with an about two fold factor
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32 14 compared to the expected level.

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35 15 Therefore, absence of relationships between DON contamination of flours and semolina and the
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37 16 ash and phytic acid contents, as well as evidence of DON concentration in the finest particles from
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39 17 the endosperm (Table 5) could explain the unexpected distribution of DON in the milling fractions.
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41 18 It thus revealed that contamination of semolina or flours could not only be originated from the grain
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43 19 outer layers but also from other contaminated parts of the grain that were shown to be friable. It
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45 20 appeared particularly significant for grains containing a high level of mycotoxin and for fractions
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47 21 coming out from the first steps of the process. Thus, higher DON level in the finest particles from
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49 22 the semolina fraction of the most infected wheat sample could result from a deeper fungi
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51 23 penetration in grains or a higher damage of the grain internal structure due to an increased synthesis
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53 24 of hydrolytic enzymes, that are known to be produced by *Fusarium* (Jackowiak et al 2005).
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55 25 However, further works are needed to test this hypothesis and examine if it is also observed for
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57 26 other durum wheat cultivars. Nevertheless, these studies point out the interest to control the
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2 1 generation and distribution of the finest particles during milling, in order to lower the DON amount
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4 2 in semolina. This could be potentially achieved by improving the sizing efficiency of the sifters
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6 3 after the two first break rolls or by increasing the efficiency of the first two sizing purifiers in order
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8 4 to better eliminate the finest particles at the first milling steps even if this will results in a decrease
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10 5 of the semolina yield. Indeed as semolina are the fractions used for pasta making, this control could
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12 6 constitute a key factor in order to reduce the DON level in corresponding consumed wheat products.
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18 8 **Acknowledgments**

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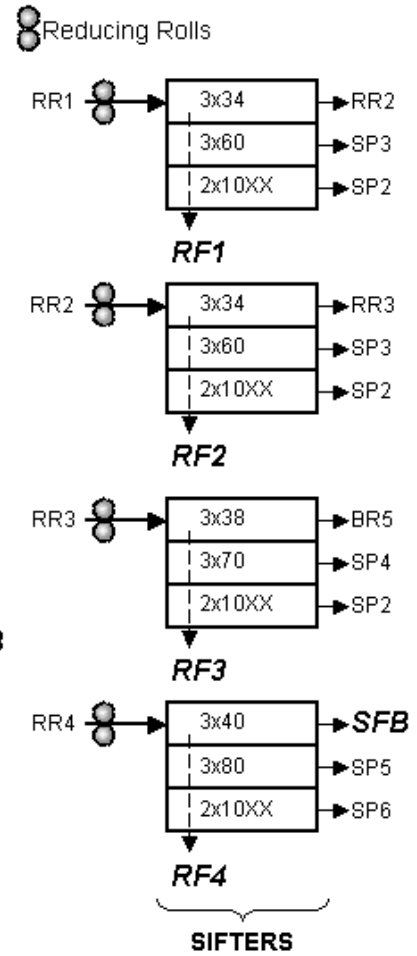
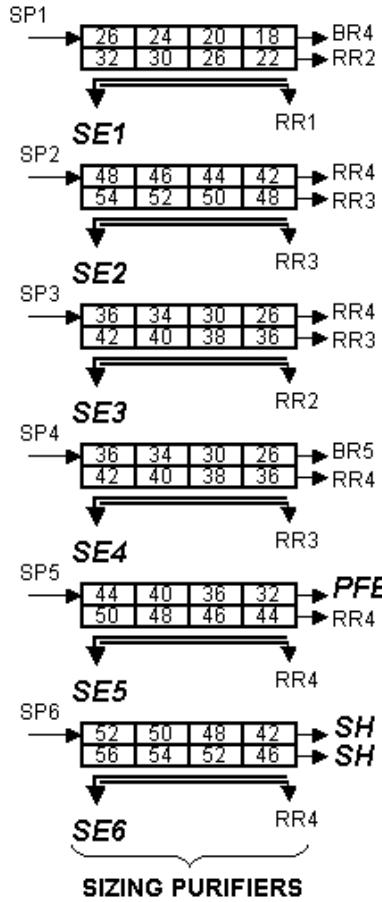
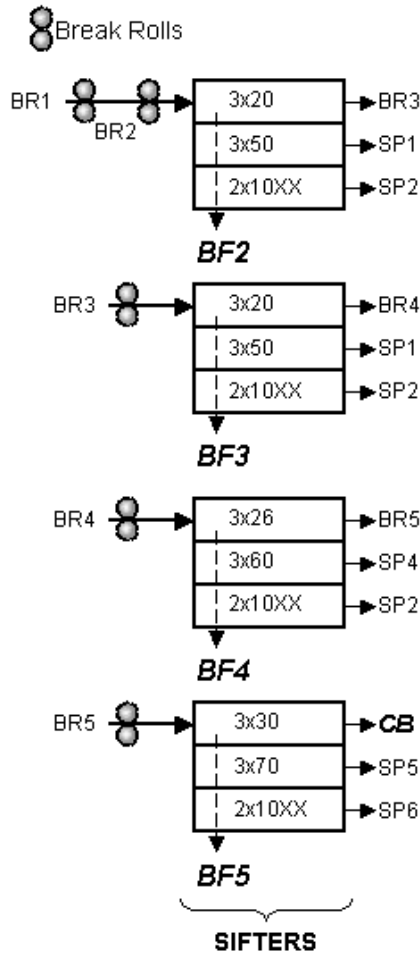
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2 1 **Figure captions**

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4 2
5 3 **Figure 1:** Milling diagram of the semi industrial pilot mill (INRA, Montpellier). BR1 to BR5 are
6 4 feedings of break rolls, BF2 to BF5 are corresponding break flours, SP1 to SP6 are feedings of
7 5 purifiers, SE1 to SE6 are semolina; RR1 to RR4 are feedings of reduction rolls, RF1 to RF4 are
8 6 reduction flours, CB, PFB and SFB are respectively coarse bran, purified fine bran and sized fine
9 7 bran, SH are shorts.

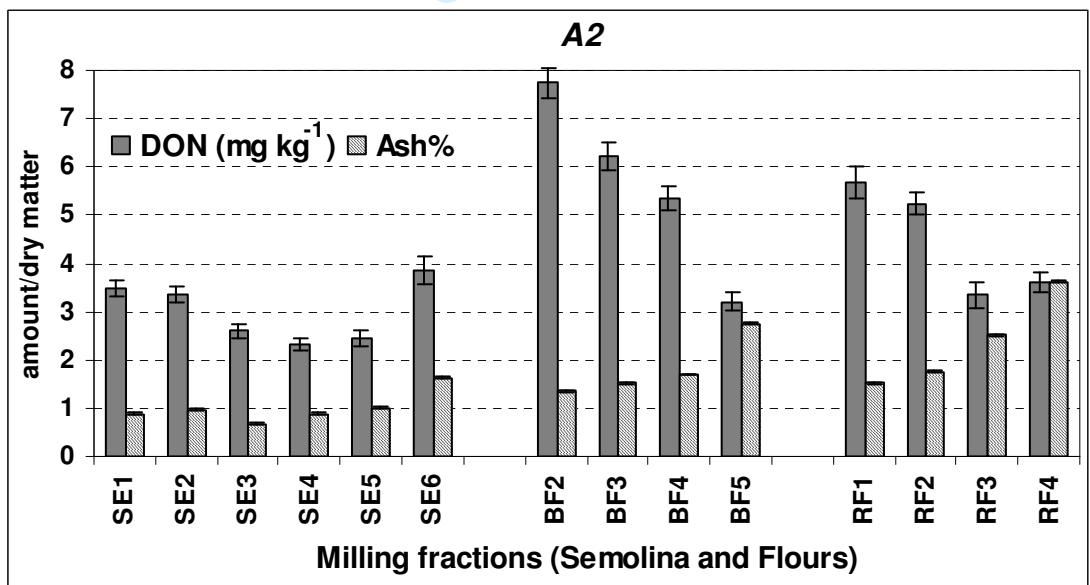
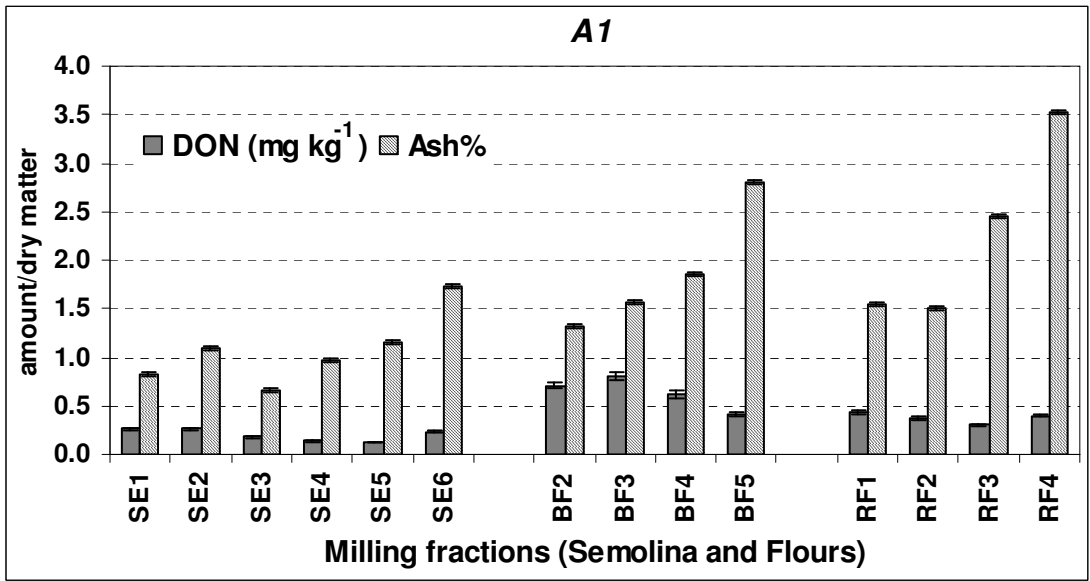
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11 9 **Figure 2:** Comparison of DON (mg.kg^{-1} d. m.) and ash (% d. m.) concentration in the milling
12 10 fractions (except brans and shorts) from the two durum wheat samples (means of two replicates).

13 11
14 12 **Figure 3 :** Detection of TCT-producing fungi in grains and milling fractions from durum wheat
15 13 *Acalou* samples A1 and A2 after PCR with primers derived from the gene encoding trichodiene
16 14 synthase. *F. graminearum* corresponds to DNA extracted from the fungus. ST are total semolina,
17 15 SE1 and SE3 are semolina from the most inner part of the endosperm, BF2 are the first break flours,
18 16 RF1 are the first reduction flours, CB, SH, SFB and PFB are respectively coarse bran, shorts, sized
19 17 fine bran and purified fine bran.

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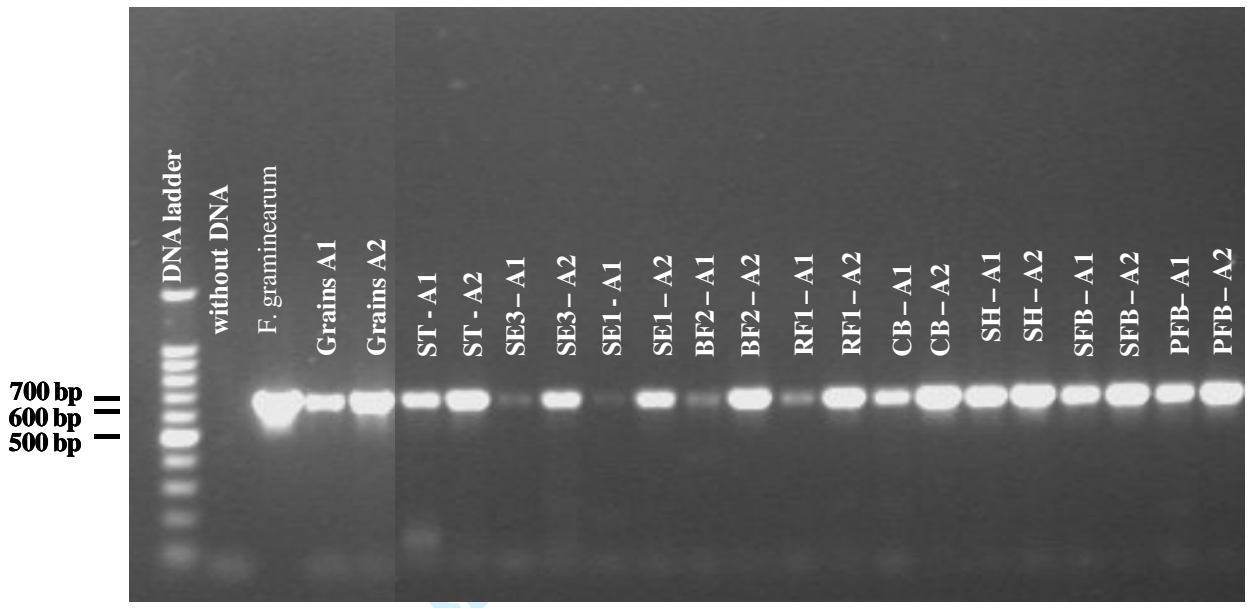


Table 1: Main physical characteristics and total DON concentration of the analyzed *Acalou* wheat samples naturally infected with *Fusarium*^a.

| Samples | A1 | A2 |
|--|------------------|--------------------|
| DON ($\mu\text{g}\cdot\text{kg}^{-1}$ d. m.) | 382.0 ± 9.7 | 4203.5 ± 202.7 |
| Test weight ($\text{kg}\cdot\text{hL}^{-1}$) | 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 | <i>A1</i> | | | | <i>A2</i> | | |
|-----------------------|---------------------------------|---|--------------------|---------------------------------|---|--------------------|-------------|
| | Yield ^a (% d. m.) | Concentrations | | Yield ^a (% d. m.) | Concentrations | | |
| | | DON ^b ($\mu\text{g.kg}^{-1}$ d.m.) | Ratio ^c | | DON ^b ($\mu\text{g.kg}^{-1}$ 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 |
| <i>Total semolina</i> | | <i>76.4</i> | <i>220</i> | <i>0.58</i> | <i>75.9</i> | <i>2940</i> | <i>0.70</i> |
| 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 |
| 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 | <i>A1</i> | | <i>A2</i> | |
|------------------------|--------------------------------|------------------------------|--------------------------------|------------------------------|
| | 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 ($\mu\text{g}\cdot\text{kg}^{-1}$ 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 | | <i>A1</i> | | <i>A2</i> | |
|-------------------------|--------------------------|------------------|-------|------------------|-------|
| | | 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 |
| 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 ($\mu\text{g.kg}^{-1}$ 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 | | | | a2 | | | |
|---|-------------------|-----------------------------------|---------|---|-------------------|-----------------------------------|---------|
| <i>SE3 A2</i> (2600 $\mu\text{g.kg}^{-1}$ d.m.) | | | | <i>SE3 A2</i> (2300 $\mu\text{g.kg}^{-1}$ d.m.) | | | |
| Sieve mesh size (mm) | Dry mass (% d.m.) | DON ($\mu\text{g.kg}^{-1}$ d.m.) | DON (%) | Sieve mesh size (mm) | Dry mass (% d.m.) | DON ($\mu\text{g.kg}^{-1}$ d.m.) | DON (%) |
| > 0.560 | 8 | 2089 | 7 | > 0.200 | 26 | 967 | 11 |
| 0.560- 0.450 | 31 | 2285 | 28 | 0.100 - 0.200 | 28 | 1555 | 19 |
| < 0.450 | 61 | 2716 | 66 | < 0.100 | 43 | 3682 | 69 |

| b1 | | | | b2 | | | |
|---|-------------------|-----------------------------------|---------|---|-------------------|-----------------------------------|---------|
| <i>SE1 A2</i> (3496 $\mu\text{g.kg}^{-1}$ d.m.) | | | | <i>SE1 A2</i> (3189 $\mu\text{g.kg}^{-1}$ d.m.) | | | |
| Sieve mesh size (mm) | Dry mass (% d.m.) | DON ($\mu\text{g.kg}^{-1}$ d.m.) | DON (%) | Sieve mesh size (mm) | Dry mass (% d.m.) | DON ($\mu\text{g.kg}^{-1}$ d.m.) | DON (%) |
| > 0.630 | 24 | 2418 | 18 | > 0.315 | 31 | 1087 | 11 |
| 0.630 – 0.560 | 32 | 2956 | 29 | 0.160 - 0.315 | 30 | 1863 | 18 |
| < 0.560 | 44 | 3912 | 53 | < 0.160 | 39 | 5736 | 71 |