



HAL
open science

THE FATE OF TRICHOTHECENE MYCOTOXINS DURING THE PROCESSING: MILLING AND BAKING

Katerina Lancova, Jana Hajslova, Marta Kostelanska, Monika Sehnalova, Jan Nedelnik, Hana Moravcova, Marie Vanova

► **To cite this version:**

Katerina Lancova, Jana Hajslova, Marta Kostelanska, Monika Sehnalova, Jan Nedelnik, et al.. THE FATE OF TRICHOTHECENE MYCOTOXINS DURING THE PROCESSING: MILLING AND BAKING. *Food Additives and Contaminants*, 2008, 25 (05), pp.650-659. 10.1080/02652030701660536 . hal-00577430

HAL Id: hal-00577430

<https://hal.science/hal-00577430>

Submitted on 17 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



THE FATE OF TRICHTHOCENE MYCOTOXINS DURING THE PROCESSING: MILLING AND BAKING

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2007-228.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	20-Aug-2007
Complete List of Authors:	Lancova, Katerina; Institute of Chemical Technology, Prague, Department of Food Chemistry and Analysis Hajslova, Jana; Institute of Chemical Technology, Prague, Department of Food Chemistry and Analysis Kostelanska, Marta; Institute of Chemical Technology, Prague, Department of Food Chemistry and Analysis Sehnalova, Monika; Institute of Chemical Technology, Prague, Department of Food Chemistry and Analysis Nedelnik, Jan; Research Institute for Fodder Crops Moravcova, Hana; Research Institute for Fodder Crops Vanova, Marie; Agricultural Research Institute
Methods/Techniques:	Chromatography - LC/MS
Additives/Contaminants:	Mycotoxins - trichothecenes
Food Types:	Bakery products, Bread, Cereals and grain, Processed foods

SCHOLARONE™
Manuscripts

1
2
3 **THE FATE OF TRICHOHECENE MYCOTOXINS DURING THE PROCESSING:**
4
5 **MILLING AND BAKING**
6
7
8
9

10 **ABSTRACT**
11

12
13
14 Toxic secondary metabolites produced by fungi representing *Fusarium* genus are common
15 contaminants in cereals worldwide. To estimate the dietary intake of these trichothecene
16 mycotoxins, information on their fate during cereal processing is needed. Up-to-date
17 techniques such as HPLC coupled with tandem mass spectrometry (LC-MS/MS), was used
18 for the analysis of seven trichothecenes (deoxynivalenol, nivalenol, HT-2 toxin, T-2 toxin, 15-
19 and 3-acetyldeoxynivalenol and fusarenon-X) in bread production chain (wheat grains,
20 intermediate products collected during milling and baking process, breads). Regardless of
21 whether the grains were naturally infected or artificially inoculated by *Fusarium* sp. in the
22 field, the fractions obtained from the grain cleaning procedure contained the highest
23 mycotoxin levels. During milling, the highest concentrations of DON were found in the bran,
24 the lowest in the reduction flours. Baking at 210°C for 14 min had no significant effect on
25 DON levels. The rheological properties of dough measured by fermentograph, maturograph,
26 oven rise recorder and laboratory baking test were carried out and based on obtained results
27 the influence of mycotoxin content on rheological behaviour was investigated.
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

INTRODUCTION

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

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

1
2
3 To protect consumers from unacceptably high dietary intake of trichothecenes, maximum
4 levels have been set in EU legislation (Commission regulation No 1881/2006, 2006).
5
6 Currently, maximum levels are regulated in cereals (1250 µg/kg), flours (750 µg/kg) and
7
8 bread (500 µg/kg) for deoxynivalenol (DON) only. Nevertheless, regulation for another two
9
10 toxic trichothecenes, HT-2 toxin (HT-2) and T-2 toxin (T-2), is under preparation.
11
12
13
14

15
16
17 The fate of DON, the most frequent *Fusarium* toxin during various processing practices has
18 been documented in several studies. Experiments concerned with distribution of DON among
19
20 the milling fractions in naturally *Fusarium* contaminated wheat showed that the highest
21
22 concentration of this mycotoxin is in the bran layer while in white flour, compared to whole
23
24 grains, is reasonably lower (Tanaka et al. 1986, Abbas et al. 1985). In another milling study
25
26 (Trigo-Stockli et al, 1996), the white flour had approximately half the level of DON in the
27
28 cleaned wheat, whilst the bran contained levels two or more times higher than the wheat.
29
30 However, the effects of yeasts on DON levels in dough during the baking fermentation step
31
32 are rather contradictory. Young et al. (1984) observed an increase of DON in yeasted products
33
34 while on the other hand, a mean reduction of DON in fermented dough of over 20% was
35
36 documented (Neira et al, 1997). The influence of various ingredients added to dough before
37
38 baking on the DON levels in bread was studied by Boyacioglu et al. (1993). Compared to
39
40 control samples, a reduction of DON as high as 40% occurred in bread due to the addition of
41
42 sodium bisulphite, L-cysteine and ammonium phosphate in to the dough. However, other
43
44 additives such as potassium bromate and L-ascorbic acid were ineffective in this context.
45
46
47
48
49
50
51
52
53
54

55 In addition to health risks associated with the presence of trichothecenes in the food supply,
56
57 other negative aspects have to be considered. The relationship between rheological properties
58
59 of dough and extent of *Fusarium* infection in wheat has been discussed in the study of Dexter
60

1
2
3 et al. (1996). Lower water absorption of flour prepared from *Fusarium* infected grains, shorter
4
5 development time and lower stability of dough, which also became progressively stickier and
6
7 difficult to handle during sheeting and moulding, were described.
8
9

10
11
12 The objective of the present study was to evaluate the effects of wheat processing, milling and
13
14 baking, on the transfer of mycotoxins into products such as flour and fermented bread. As in
15
16 the case of other contaminants, the knowledge of the fate of mycotoxins during processing is
17
18 important both for dietary intake estimation and adopting measures for its minimisation. In
19
20 addition to these aspects, the effect of mycotoxin contamination on the dough rheological
21
22 properties was determined.
23
24
25
26
27
28

29 MATERIALS AND METHODS

30 *Samples*

31 *Wheat grains*

32
33
34 Four grain samples (each 5000 g) obtained from wheat grown under well characterised
35
36 conditions (Table I) in the year 2005 were supplied by Agricultural Research Institute in
37
38 Kromeriz, the Czech Republic. Three of these samples (A, B, C) were naturally infected with
39
40 *Fusarium* species; sample (D) was spray-inoculated in the field with a spore-mixture of
41
42 *Fusarium culmorum* and *F. graminearum* (1:1; 10^6 conidia/ml suspension; 250-300 l/ha) at
43
44 the time of flowering. The levels of trichothecenes in the grains used for the processing
45
46 experiments were determined by LC/MS-MS and shown in Table I.
47
48
49
50
51
52
53
54

55 *Processed wheat-based products*

56 *Milling fractions*

57
58
59
60

1
2
3 Before milling with an automatic laboratory mill (model MLU-202, Bühler), grain samples
4 were cleaned (sieving, scouring and polishing). During grains cleaning, screenings, the waste
5 obtained by sieving process (the size of sieve was 2.2 mm; the laboratory screening machine
6 in-house made prototype) and outer layers of bran, the combined waste from the scouring and
7 polishing (the laboratory scouring in-house made prototype; the laboratory aspirator of dust
8 particles Labofix Brabender) were collected. Milling fractions (three break flours fractions,
9 three reduction flours fractions and bran fractions), according to the flow chart (Figure 1),
10 were examined for their content of trichothecenes. The sizes of used sieves are shown in
11 Figure 1. Weight proportions of wheat fractions and their moistures are reported in Table II.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26

27 *Bread*

28
29 The process of bread making is outlined in Figure 2. The main components of white flour
30 were prepared by the mixing of all three break flours and the 1st reduction flours obtained
31 from grains A – D according to schema in Figure 1 (for weight see Table II). For the
32 preparation of one batch of breads, 300 g of white flour was taken and the following
33 ingredients were added: yeast *Saccharomyces cerevisiae* (12 g), saccharose (5.1 g), vegetable
34 oil (4.5 g), salt (2.1 g), distilled water (150 – 165 ml; with the volume varying according to
35 bonding power). Immediately after kneading the dough was placed in a covered bin
36 (container) containing a laboratory thermostat and allowed to ferment at 30°C for 50 minutes.
37
38 The dough was then divided into pieces of approx. 70 g each and moulded by hand. The final
39 proof of the dough pieces proceeded at the same temperature (30°C) as during the stage of
40 fermentation for 45 min. The loaves of bread were baked in an electric laboratory oven at
41 240°C for 14 min. All intermediate products prepared within the baking process were dried
42 for 24 hour at 40°C prior to analysis.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Evaluation of rheological properties

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

Trichothecene analysis

LC/MS/MS) was used for the analysis of 7 trichothecene mycotoxins in sample extracts purified by solid phase extraction (SPE). Wheat grains (raw materials), milling fractions and intermediates collected during baking as well as the resulting breads.

Standards

Trichothecene standards (deoxynivalenol, DON; nivalenol NIV; HT-2 toxin, HT-2; T-2 toxin, T-2; 15- and 3-acetyldeoxynivalenol, ADONs and fusarenon-X, Fus-X) were purchased from Sigma-Aldrich (Germany) and Biopure (Austria). Certified reference materials, “DON in wheat flour” (< 0.05 mg/kg, BCR 396, Belgium) and “DON in naturally contaminated wheat” (0.7 ± 0.1 mg/kg, R-Biopharm Rhone, UK), were used as controls to ensure the accuracy of measurements. The values obtained for DON were in the uncertainly range of certificate concentrations.

Extraction and clean-up procedure

1
2
3 Representative samples (12.5 g) was extracted with 50 ml acetonitrile:water mixture
4 (84:16,v/v) for one hour using an automatic shaker (IKA Laborortechnik, Germany). Crude
5 extracts were then filtered (Filtrak No. 390, VEB Freiburger, Germany) and 8 ml aliquots
6 transferred into sample tubes to which 80 µl of acetic acid (99%, Sigma-Aldrich) were added.
7
8 Purification was achieved by solid phase extraction (SPE) employing the MycoSep™ 226
9 cartridges (Romer, Austria). Four ml of purified extract were evaporated to dryness and the
10 residues transferred into 1 ml of a water:methanol mixture (80:20, v/v) and passed through a
11 0.2 µm microfilter (Alltech, USA) prior to further analysis.
12
13
14
15
16
17
18
19
20
21

22 *Chromatographic conditions*

23
24 High performance liquid chromatography (HP1100 Binary Series LC system, Agilent
25 Technologies, USA) coupled with a mass spectrometer (Finnigan LCQ Deca, USA) were
26 used for the analysis of purified extracts. Chromatographic separation of sample components
27 was carried out on a reverse phase column with polar endcapping (Synergi Hydro RP, 150mm
28 x 3mm x 4µm, Phenomenex, USA) heated at 40 °C and operated under gradient conditions.
29 The mobile phase was composed of 10 mM ammonium acetate in purified water (A) and
30 methanol (B). The flow rate of mobile phase was set to 0.5 ml/min and the injection volume
31 was 20 µl. Gradient elution was performed starting from A:B (80:20, v/v) and reaching A:B
32 (30:70, v/v) in 8 min. From 8 to 15 min the ratio A:B (30:70, v/v) was stable and then jumped
33 to A:B (80:20, v:v). The time of post run lasted 7min.
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

50 Identification and quantification of analytes was performed using MS/MS with the following
51 parameters (ion source type – APCI operated both in negative and positive ion modes,
52 capillary temperature - 150 °C, vaporizer temperature - 450 °C, nitrogen sheath gas flow – 1.2
53 l/min, nitrogen auxiliary gas flow – 3 l/min, source voltage - 6 kV, collision gas - helium,
54 scan type - selected reaction monitoring). APCI ionization modes (⁺/⁻) and monitored
55
56
57
58
59
60

1
2
3 fragments m/z (parent ion>daughter ion, confirmation ion) used for individual analytes were
4
5 DON⁻ (371>311, 281), NIV⁻ (355>295, 265), FUS-X⁻ (413>353, 263), ADONs⁻(397>337,
6
7 307), HT-2⁺(442>425, 263) and T-2⁺(317>273, 299).
8
9

10 *Performance characteristics of analytical method / Quality assurance*

11
12 Limits of detection (LODs) and quantification (LOQs), recoveries and repeatabilities
13
14 expressed as relative standard deviations (RSDs) that were obtained within the validation
15
16 process are reported in Table III. Calibration curves for all analytes were linear within the
17
18 working range from 5 to 10 000 µg/kg. Squared correlation coefficients (R²) were in the range
19
20 0.9991 – 0.9999 for 11 point calibration curves.
21
22
23
24
25
26

27 The analytical method used for samples examination was accredited (ISO 17025) for cereals;
28
29 as a part of external quality control the trueness of generated data was demonstrated through
30
31 participation in Food Analysis Performance Assessment Scheme (FAPAS) organized by
32
33 Central Science Laboratory (CSL, York UK). The z-scores for all analysis (DON, ZON, HT-2
34
35 and T-2) were in the range ±2.
36
37
38
39
40

41 *Moisture determination*

42
43 Moisture was determined gravimetrically by drying samples in an oven for 2 hours at 131 ± 2
44
45 °C according to ISO Standard No. 712 “Moisture determination in cereals and cereal
46
47 products”.
48
49
50
51
52

53 **RESULTS AND DISCUSSION**

54
55 In common with some other contaminants, the levels of mycotoxins in processed foods may
56
57 differ considerably from those in raw materials. Depending on the household and/or industrial
58
59 processing practise, both reduction and accumulation of toxins may occur in a particular
60

1
2
3 product. In any case, these changes should be taken into account when estimating consumer
4 exposure on the basis of data obtained by analysis of cereal grains.
5
6

7 *Distribution of Fusarium mycotoxins in wheat grains*

8
9 For processing experiments, four wheat grain samples, representing various contamination
10 characteristics (both in terms of total content of trichothecenes and their patterns) were used.
11
12

13 A fairly uneven distribution of trichothecenes within the fractions obtained during the milling
14 process was apparent and is shown in Table IV. Relatively very high contents of
15 trichothecenes were found in waste fractions obtained within cleaning process. Effective
16 removing of all screenings and outer layers of bran from the surface of grains during cleaning
17 steps reduced DON content by 50, 55, 41 and 47% in cleaned wheat samples A, B, C and D,
18 respectively. The contents of DON in individual milling fractions are shown in Figure 3.
19
20

21 Considering the weight contribution of various flour fractions to the total mass of cleaned
22 grains taken for milling, most of DON (19, 17, 21 and 20% in A, B, C and D samples,
23 respectively) was contained in the 1st reduction flours because of high DON levels and
24 amount of this type of milling fraction. Very similar results were reported by Japanese
25 researchers (Tanaka et al., 1986) who used the same type of laboratory mill for fractionation
26 of naturally contaminated wheat. Generally, the data obtained by different mill might not be
27 comparable.
28
29

30 *Changes of DON concentration during baking process*

31
32 Four white flours prepared by mixing respective break flours and the 1st reduction flours (see
33 Figure 1), were used for baking experiments. DON levels in these flours corresponding to
34 grains A, B, C and D were 413, 52, 40 and 1223 µg/kg, respectively. Other target
35 trichothecene mycotoxins (NIV, ADONs, Fus-X, HT-2 and T-2) were not detected in any of
36 the flour samples. The changes in DON concentrations during dough processing (kneaded,
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 fermented and final proof dough) and in the final bread are illustrated graphically in Figures 4
4 – 7 and summarised in Table V. Considering the total amount of DON contained in white
5 flours which were taken for breads production, its levels were higher by 21 – 40% in kneaded
6 dough, however during fermentation, a significant decrease occurred of approximately 38 –
7 46% of the original content (the calculation, i.e. basis for flour amount in the same in all these
8 products). Surprisingly, the dough pieces at the end of proofing phase contained relatively
9 high DON levels, ranging from 132-145% of the total flour content in products A, B, C and
10 D. However, when comparing the content of DON in the raw materials (flours) and final
11 products (loaves), practically no changes occurred with a maximum loss of 6% in bread
12 relevant to sample D. In spite of apparently unaffected DON levels in the dough processing is
13 accompanied with distinct fluctuation of its contents. Release of DON from conjugated form
14 probably is followed by its transformation into a substance that cannot be determined by
15 conventional analysis, unfortunately, its nature remains unclear. It is worth noting that the
16 same profile of changes in DON levels occurred in all samples regardless of the extent of
17 contamination. Remarkably a temporary drop of DON (approximately 40%) as the result of
18 dough fermentation was also observed in a reference earlier studies. Overall, the DON content
19 in loaves was practically the same as that determined in white flours taken for their
20 preparation. In other words, no mitigation of this mycotoxin occurred during bread-making
21 process. This conclusion complies with results obtained in several studies carried out in
22 various countries, see Table VI. Recently, many discussions on masked mycotoxins in cereals
23 have been opened. Deoxynivalenol-3-glucoside has been shown to be the most abundant
24 DON derivative (Berthiller, 2005). Unfortunately, it was beyond the scope of this study to
25 search for DON conjugates. In any case, the efficiency of extraction process was tested for
26 each baking intermediates. Hence it seems unprovably that drops of DON content were due to
27 its bounding by the yeast.
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 *Effect of mycotoxin content on rheological properties*
4

5 As mentioned earlier, besides of health risk associated with infection of cereals by *Fusarium*
6 fungi, other problems corresponding to the quality of flour may be encountered. Two
7
8 important fermentographic characteristics, dough volume and time of fermentation, describe
9
10 properties and behaviour of dough before the collapse of its structure caused by biochemical
11
12 changes within fermentation. The values of the two fermentographic factors mentioned above
13
14 in wheat samples A, B, D were higher approximately by 40% than in sample C (the lowest
15
16 DON level), see Table VII.
17
18
19
20
21

22
23
24 Maturograph characteristics belong to the most important parameter in description of
25
26 rheological dough properties. Proofing time ensures the optimum time for reaching the
27
28 maximum volume of final bakery products. It was clear, that dough prepared from infected
29
30 grains needed a longer period for approaching this optimal time. Dough resistance against
31
32 mechanical stress during proofing correlated with DON content with the higher contamination
33
34 in dough giving the higher dough resistance. On the other hand, the elasticity of fermented
35
36 dough was not affected by extent of contamination. Proofing stability of dough reflects the
37
38 time tolerance of optimal proofing and so ensures the highest volume of the final products.
39
40 Regarding this parameter, the dough stability prepared from the sample contaminated with the
41
42 lower amount of DON (Sample C) was twice as high in comparison with the flour
43
44 contaminated with the most DON (Sample D).
45
46
47
48
49
50

51
52 The values of baking volume recorded by oven rise recorder, which indicates the final volume
53
54 of baked products, were slightly higher for samples containing mycotoxins. This trend was
55
56 found also for another rheological factor, oven rise that characterises difference in volume
57
58 between baked product and unprocessed dough.
59
60

CONCLUSIONS

The results obtained within this study can be summarised as follows:

- i) The highest levels of both type B trichothecenes (deoxynivalenol, nivalenol, sum acetyl-DON) and type A trichothecenes (HT-2 and T-2 toxin) were concentrated in waste fractions (screenings, outer layers of bran) obtained during cleaning of wheat grains. On this occasion, a potential health risk for staff breathing these materials at gristmills should be mentioned.
- ii) A substantial part of DON in cleaned grains is located in the bran. Nevertheless, approximately 40% of its original content was still left in flour fractions; the 1st break flour was the most contaminated.
- iii) Pronounced changes of DON levels in dough samples taken in particular steps of baking process might be due to transformation of DON forms (free / conjugate). More research is needed to explain these phenomena.
- iv) No significant reduction of free DON levels occurred as the result of bread baking process (210°C, 14 min).
- v) Some rheological properties such as proofing time and dough stability were found to be worse in samples prepared from more DON contaminated grains, nevertheless more than DON level, the overall, impact of the mould growth on the rheological properties is important.

ACKNOWLEDGEMENTS

This research was supported by the National Agency of Agricultural Research (NAZV) of the Czech Republic, project no. QF3121 (1Q57042). Part of funding was obtained from project MSM 6046137305 granted by the Ministry of Education, Youth and Sports of the Czech

1
2
3 Republic. Thanks for providing valuable advices and carrying out the milling experiments
4
5 belong to Oldrich Famera, MSc. from the Czech University of Life Sciences Prague and the
6
7 bakery experiments belong to Assoc. Prof. Marie Hrusková from the Department of
8
9 Carbohydrate Chemistry and Technology (ICT Prague).
10
11

12 13 14 15 REFERENCES

16
17 Abbas HK, Mirocha CJ, Pawlosky RJ, Pusch DJ. 1985. Effect of cleaning, milling and baking
18
19 on deoxynivalenol in wheat. *Applied and Environmental Microbiology* 50:482 – 486.
20

21
22 Berthiller F., Dall'Asta ch., Schuhmacher R., Lemmens M., Adam G., Krska R., 2005,
23
24 Masked Mycotoxins: Determination of a Deoxynivalenol Glucoside in Artificially and
25
26 Narturally Contaminated Wheat by Liquid Chromatography-Tandem Mass Spectrometry.
27
28 *Journal of Agricultural and Food Chemistry* 53, 3421-3425.
29

30
31 Boyacioglu D, Hettiarachcy NS, D'Appolonia BL. 1993. Additives affect deoxynivalenol
32
33 (Vomitoxin) flour during breadmaking. *Journal of Food Science* 58 (2):416 – 418.
34

35
36 Commision Regulation (EC) No 1881/2006 of 19 December 2006. Official Journal of the
37
38 European Union L364:7-8.
39

40
41 Creppy EE. 2002. Update of survey, regulation and toxic effect of mycotoxins in Europe.
42
43 *Toxicology Letters* 127:19-28.
44

45
46 Dexter JE, Clear RM, Preston KR. 1996. Fusarium head blight: Effect on the milling and
47
48 baking of some Canadian wheat. *Cereal Chemistry* 73 (6):695 – 701.
49

50
51 D'Mello JPF, Macdonald AMC. 1997. Mycotoxins. *Animal Feed Science Technology* 69:155-
52
53 166.
54

55
56 Edwards SG. 2004. Influence of agricultural practices on fusarium infection of cereals and
57
58 subsequent contamination of grain by trichothecene mycotoxins. *Toxicology Letters* 153:29-
59
60 35.

1
2
3 Hazel CM, Patel S. 2004. Influence of processing on trichothecene levels. *Toxicology Letters*
4
5 153:51 – 59.
6

7
8 Kuiper-Goodman T. 2004. Risk assessment and risk management of mycotoxins in food. In:
9
10 Magan N., Olsen M., editors. *Mycotoxins in food*. England: Woodhead Publishing Limited. p
11
12 3.
13

14
15 Lee US, Jang HS, Tanaka T, Oh YJ, Cho ChM, Ueno Y. 1987. Effect of Milling on
16
17 Decontamination of *Fusarium* Mycotoxins Nivalenol, Deoxynivalenol, and Zearalenone in
18
19 Korean Wheat. *Journal of Agricultural and Food Chemistry* 35:126.
20

21
22 Neira MS, Pacin AM, Martínez EJ, Moltó G, Resnik SL. 1997. The effects of bakery
23
24 processing of natural deoxynivalenol contamination. *International Journal of Food*
25
26 *Microbiology* 37:21 - 25.
27

28
29 Prange A, Birzele B, Kramer J, Meier A, Modrow H, Kohler P. 2005. *Fusarium*-inoculated
30
31 wheat: deoxynivalenol contents and baking quality in relation to infection time. *Food Control*
32
33 16:739-745.
34

35
36 Scudamore KA, Banks JN, MacDonald SJ. 2003. The fate of ochratoxin A in the processing
37
38 of whole wheat grains during milling and bread production. *Food Additives and*
39
40 *Contaminants* 20 (12):1153-1163.
41

42 Schlatter J., 2004. Toxicity data relevant for hazard characterization. *Toxicology Letters* 153,
43
44 83-89.
45

46 Tanaka T, Hasegawa A, Yamamoto S, Matsuki Y, Ueno Y. 1986. Residues of *Fusarium*
47
48 mycotoxins, nivalenol, deoxynivalenol and zearalenone in wheat and processed food after
49
50 milling and baking. *Journal of the Food Hygienics Society of Japan* 27 (6):653 – 655.
51

52 Trigo-Stockli DM, Deyoe CW, Satumbaga RF, Pedersen JR. 1996. Distribution of
53
54 deoxynivalenol and zearalenone in milled fractions of wheat. *American Association of Cereal*
55
56 *Chemists* 73(3):388-391.
57

58
59 Visconti A, Haidukowski EM, Pascale M, Silvestri M. 2004. Reduction of deoxynivalenol
60
during durum wheat processing and spaghetti cooking. *Toxicology Letters* 153:181 – 189.

1
2
3 Weidernborner M. 2001. Encyclopedia of Food Mycotoxins. ISBN 3-540-67556-6. Springer-
4 Verlag Berlin Heidelberg.
5

6
7
8 Young JCH, Fulcher RG, Hayhoe JH, Scott PM, Dexter JE. 1984. Effect of milling and
9 baking on deoxynivalenol (vomitoxin) content of eastern candadian wheats. Journal of
10 Agricultural and Food Chemistry 32:659-664.
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

Figure 1 Simplified chart of milling process used for obtaining experimental samples (W = wire; SS = stainless steel)

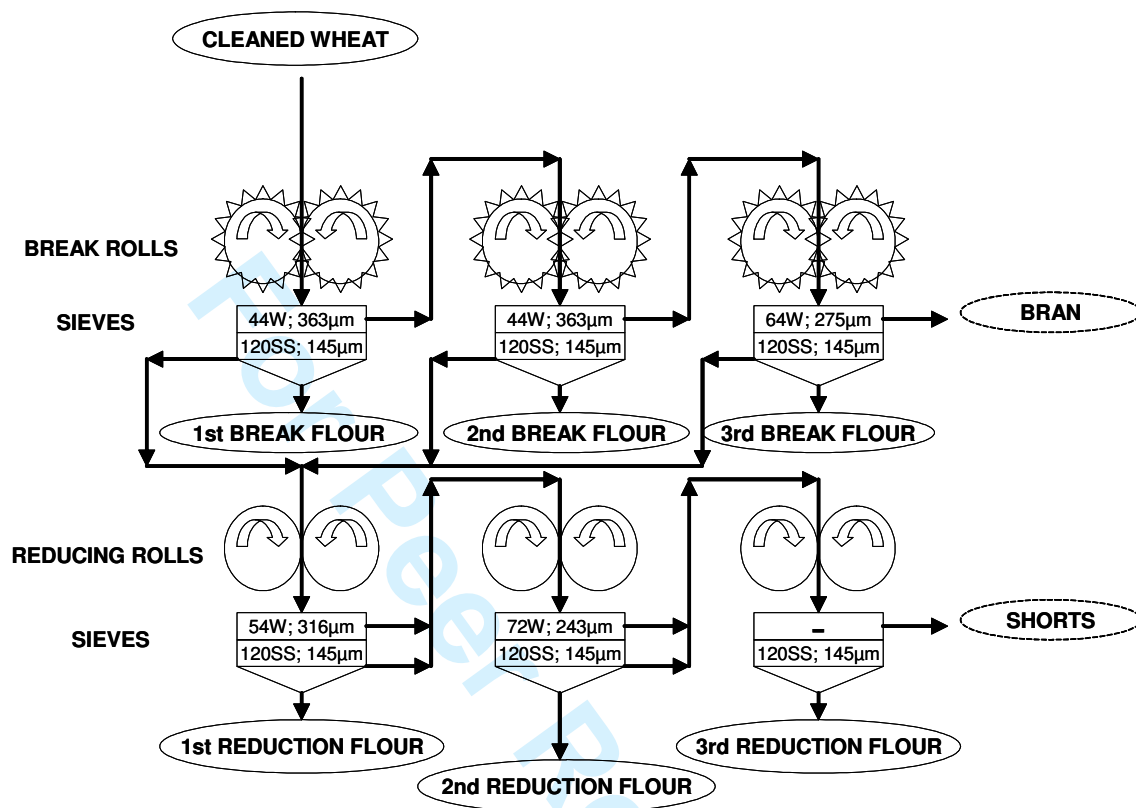


Figure 2 Bread making process

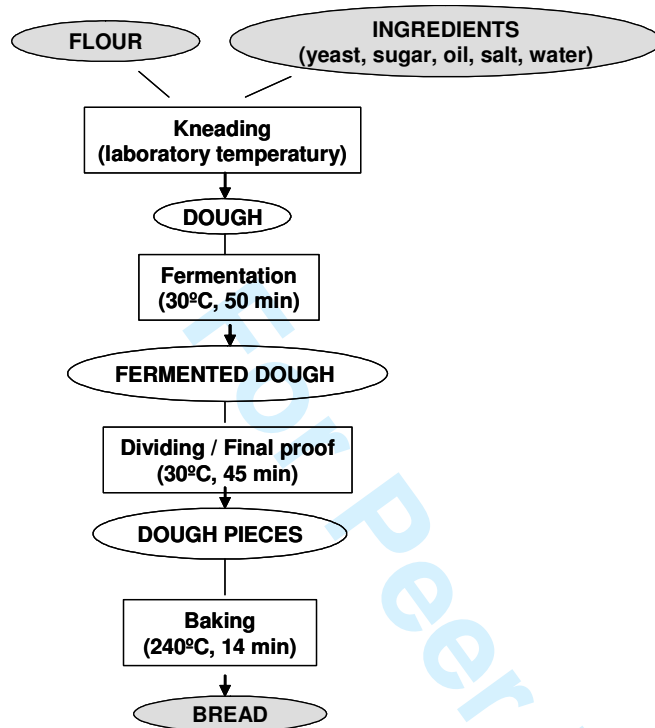
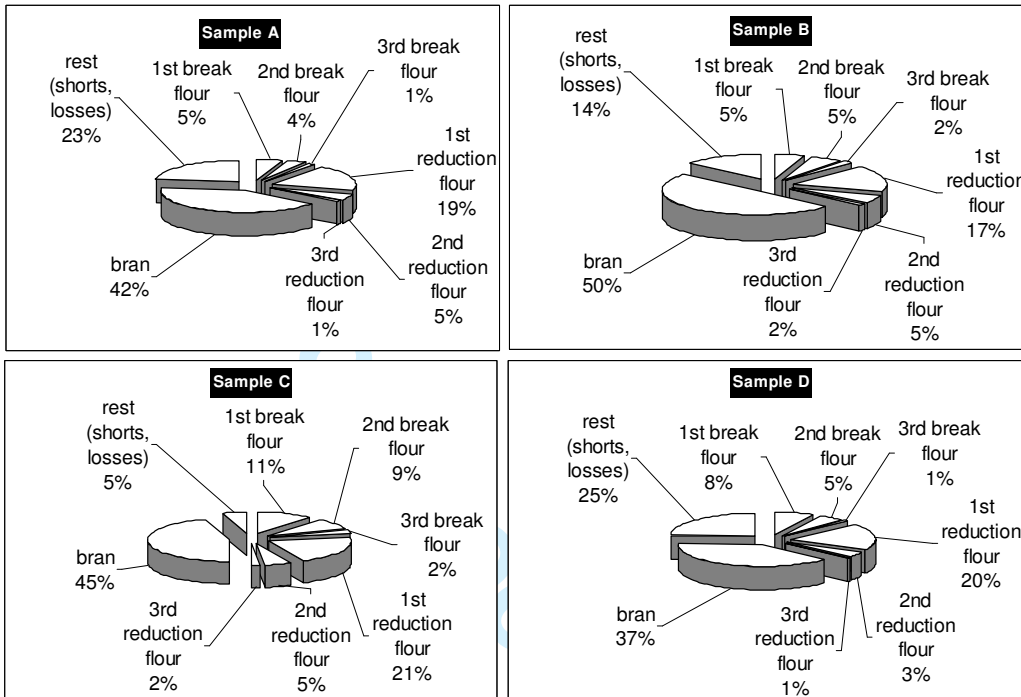


Figure 3 DON distribution (%) in milling fractions (total DON amount in of 4000 g cleaned grains = 100%; the contributions were calculated on the weight of obtained milling fractions shown in Table II) and determined concentration levels of trichothecenes in these samples summarised in Table I

Deleted: summarised



Review Only

Figures 4 - 7 DON levels in intermediate products collected during baking process (white flour = F; kneaded dough = KD; fermented dough = FD; dough after final proof = DP; bread = B), concentrations expressed on the dry weight of respective product

Deleted: <sp>

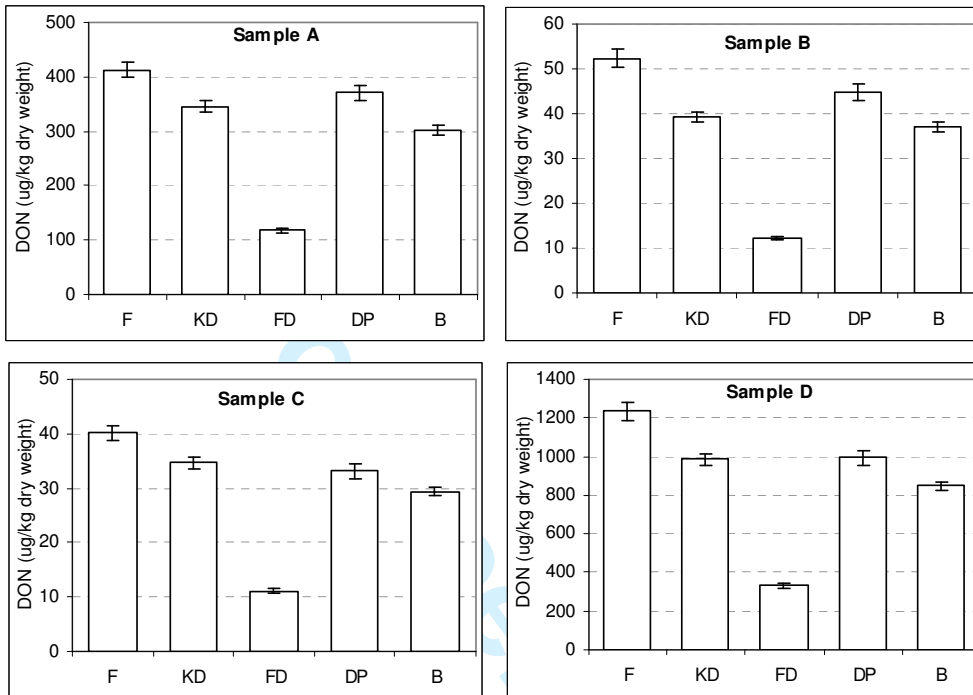


Table I Characteristics of wheat grains used for processing experiments

<i>Sample Code</i>	<i>Wheat cultivar</i>	<i>Preceding crop</i>	<i>Fusarium infection</i>	<i>Way of cultivation</i>	<i>Trichothecene levels (µg/kg)</i>					
					<i>DON</i>	<i>NIV</i>	<i>ADONs</i>	<i>Fus-X</i>	<i>HT-2</i>	<i>T-2</i>
A	Sulamit	maize	natural	conventional	909	23	35	< 1	< 10	< 5
B	Sulamit	trifolium	natural	organic	108	81	< 10	< 1	22	< 5
C	Ebi	rape	natural	conventional	92	30	< 10	< 1	< 5	< 5
D	Ebi	maize	artificial	conventional	2985	< 10	69	< 1	< 10	< 5

For Peer Review Only

Table II Characteristics of cleaning and milling fractions

Process	Fraction	Weight proportion of wheat fractions (%) / Moisture (% w/w)			
		Sample Code (see Table I)			
		A	B	C	D
Cleaning	uncleaned grain	100.0 / 13.9	100 / 13.3	100.0 / 13.5	100.0 / 13.6
	screening	3.0 / 13.6	8.8 / 12.8	5.9 / 13.1	7.8 / 13.1
	outlayer of bran	0.5 / 13.9	0.5 / 13.0	1.0 / 13.2	0.7 / 13.3
	cleaned grain	85.1 / 14.0	87.7 / 13.1	92.7 / 13.3	87.4 / 13.5
Milling	cleaned grain	100.0 / 14.0	100.0 / 13.1	100.0 / 13.3	100.0 / 13.5
	bran	19.7 / 14.2	25.1 / 13.4	19.4 / 14.4	22.0 / 14.5
	shorts	14.0 / *	5.5 / *	9.8 / *	10.1 / *
	1st reduction flour	5.8 / 13.4	4.9 / 13.1	9.1 / 14.4	7.9 / 12.4
	2nd reduction flour	6.5 / 13.2	7.5 / 13.2	9.9 / 14.2	8.1 / 14.3
	3rd reduction flour	1.8 / 12.4	1.8 / 12.3	2.4 / 12.4	1.9 / 13.0
	1st break flour	27.7 / 13.6	27.7 / 13.3	29.4 / 13.9	36.5 / 11.7
	2nd break flour	11.3 / 13.1	11.3 / 13.2	11.8 / 13.2	10.2 / 14.5
	3rd break flour	2.9 / 12.5	2.9 / 12.5	2.8 / 12.0	2.4 / 14.2

* shorts were not available for analysis

Deleted: ¶

Deleted: impurity

Deleted: 100.0

Deleted: dust

Deleted: 8.8

Deleted: 0.5

Table III Validation data obtained by LC-MS/MS method (n=5)

Deleted: v

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

*analytes' levels in spiked samples were 480 $\mu\text{g}/\text{kg}$

Table IV Distribution of trichothecene mycotoxins in milling fractions

Deleted: VI

Sample Code (see Table I)																				
A						B					C					D				
Mycotoxin content ($\mu\text{g}/\text{kg}$ dry weight)																				
Product	NIV	DON	ADONs	HT-2	T-2	NIV	DON	ADONs	HT-2	T-2	NIV	DON	ADONs	HT-2	T-2	NIV	DON	ADONs	HT-2	T-2
uncleaned grain	23	909	35	<LOQ	<LOQ	81	108	<LOQ	29	<LOQ	30	92	<LOQ	<LOD	<LOD	<LOQ	2985	69	<LOQ	<LOQ
impurity	19	1665	43	173	26	44	705	<LOQ	23	<LOQ	88	105	43	<LOD	<LOD	22	8419	139	32	11
dust	36	7175	94	145	9	245	2689	49	226	36	42	671	43	21	<LOD	69	25020	425	37	<LOD
cleaned grain	<LOD	456	<LOD	<LOQ	<LOD	<LOD	59	<LOD	<LOQ	<LOQ	16	38	<LOD	<LOD	<LOD	<LOD	1413	12	<LOD	<LOD
1st break flour	<LOD	391	<LOD	<LOD	<LOD	<LOD	64	<LOD	<LOD	<LOD	<LOD	42	<LOD	8	<LOD	<LOD	1057	<LOD	<LOD	<LOD
2nd break flour	<LOD	278	<LOD	<LOD	<LOD	<LOD	42	<LOD	<LOD	<LOD	<LOD	34	<LOD	<LOD	<LOD	<LOD	827	<LOD	<LOD	<LOD
3rd break flour	<LOD	270	<LOD	<LOD	<LOD	<LOD	49	<LOD	<LOD	<LOD	<LOD	39	<LOD	<LOD	<LOD	<LOD	786	<LOD	<LOD	<LOD
1st reduction flour	<LOD	314	<LOD	<LOD	<LOD	<LOD	32	<LOD	<LOD	<LOD	<LOD	27	<LOD	<LOD	<LOD	<LOD	764	<LOD	<LOD	<LOD
2nd reduction flour	<LOD	202	<LOD	<LOD	<LOD	<LOD	26	<LOD	<LOD	<LOD	<LOD	18	<LOD	<LOD	<LOD	<LOD	455	<LOD	<LOD	<LOD
3rd reduction flour	<LOD	170	<LOD	<LOD	<LOD	<LOD	33	<LOD	<LOD	<LOD	<LOD	23	<LOD	21	<LOQ	<LOD	396	<LOD	<LOD	<LOD
bran	<LOD	975	<LOD	78	<LOD	<LOD	117	<LOD	93	77	62	90	<LOD	<LOD	<LOD	<LOD	2451	32	<LOD	<LOD

Table VII Rheological characteristics of fermented dough

Deleted: IX

Sample Code	Fermentograph		Maturograph			Oven rise recorder	
	V _D (FeU)	T _{FER} (min)	T _{MAT} (min)	R _D (MaU)	S _{MAT} (min)	V _B (OU)	Oven rise (OrU)
A	91	60	40	820	6	620	190
B	83	57	46	725	8	490	190
C	61	44	34	715	12	480	195
D	85	59	40	880	6	595	220

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

For Peer Review Only

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

Deleted: II

<i>Sample characteristics</i>	<i>Product / Process conditions</i>	<i>DON in flour (µg/kg)</i>	<i>Remaining DON in bakery product (% of flour)*</i>	<i>Study</i>
naturally infected wheat	non-yeasted sponge 170 °C for 30 min	193	105	Tanaka et al., 1986
spiked flour (500 µg/kg)	non-yeasted sponge 170 °C for 30 min	665	101	Tanaka et al., 1986
artificial infected wheat heads	yeasted brad 220 °C for 20 min	3130	93	Boyacioglu et al., 1993
naturally infected wheat	yeasted brad 210 °C for 10 to 40 min	500 - 1000	24 - 100	Neira et al., 1997
naturally infected wheat	yeasted brad 205 °C for 30 min	4100	107	Scott et al., 1984
naturally infected wheat	Yeated brad data not available	520.9	31 - 81	Abbas et al., 1985

Deleted: flower

*DON in flour (100%)

For Peer Review Only

Table V. Effect of baking process on DON levels, content of DON in flour taken for processing = 100%

<i>Product</i>	<i>Sample Code (see Table I)</i>			
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
	<i>% of DON</i>			
kneaded dough	135	121	140	131
fermented dough	46	38	44	44
dough after final proof	145	139	134	132
bakery product	100	96	99	94

Deleted: II

Deleted: content (flour=100%)