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Occurrence of the Fusarium T-2- and HT-2-toxins in oats from cultivar studies in Germany and degradation of the toxins during grain cleaning treatment and food processing

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3 **Occurrence of the *Fusarium* T-2- and HT-2-toxins in oats from cultivar studies in**
4 **Germany and degradation of the toxins during grain cleaning treatment and food**
5 **processing***
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36 * Presented at ISM Conference 2009 held in Tulln (Austria) from 9-11 Sept. 2009.
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41 **Abstract**
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44 For the *Fusarium* trichothecene mycotoxins T-2 and HT-2 a combined (T-2+HT-2) temporary
45 Tolerable Daily Intake (tTDI) of 0.06 µg kg⁻¹ body weight per day has been proposed at European
46 level in 2001 (Opinion of the Scientific Committee on Food). In the near future, maximum levels
47 for these trichothecenes will be regulated by the European Commission as announced in EU (VO)
48 1881/2006. For the implementation of these maximum levels more data on occurrence and
49 behaviour of T-2- and HT-2-toxins in primary agricultural products as well as during cleaning
50 treatment and food processing are needed. In the current work we determined the T-2/HT-2
51 concentrations in four oat cultivars (Aragon, Dominik, Ivory, Pergamon) from 10 different
52 agricultural sites in Germany, grown in cultivar studies in 2007. The grains were de-hulled, oat
53 meal was prepared and bread with 20% oat meal and 80% wheat flour was baked. In the cereal
54 processing chain, samples were taken at various steps and subsequently analysed for their T-
55 2/HT-2 content. We employed LC-MS and an immunological screening method (ELISA) for T-
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3 2/HT-2 determination. Detection limits were between 1-10 $\mu\text{g kg}^{-1}$ in different matrices. T-2/HT-
4 2 concentrations determined by ELISA in oat samples from ten different agricultural sites in
5 Germany were between 9 and 623 $\mu\text{g kg}^{-1}$. Median and 90th percentile were 48 and 191 $\mu\text{g kg}^{-1}$ T-
6 2/HT-2, respectively. One site showed six times higher T-2/HT-2 levels than the other sites,
7 where concentrations ranged from 322 to 623 $\mu\text{g kg}^{-1}$. In 80% of the samples the cultivars
8 Pergamon and Ivory had the lowest concentration of T-2-and HT-2-toxins. Using LC-MS for T-
9 2/HT-2 determination, cleaning of the raw material did not lead to significant reductions of T-2-
10 and HT-2 levels, whereas de-hulling led to a reduction of over 90%. Boiling of oat meal produced
11 from cleaned raw material to yield "porridge" resulted in varying T-2/HT-2 levels in
12 experimental replicates. No major reduction of T-2/HT-2 levels in cooked porridge was obtained.
13 Standardized baking experiments using 20% oat meal showed that T-2-and HT-2-toxins are
14 relatively stable during the baking process, probably due to their temperature stability.
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24 **Keywords:** *cereal; oats; T-2-/HT-2-toxin; Fusarium; trichothecenes; porridge; bread*
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30 Introduction

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33 *Fusarium* spp. are phytopathogenic fungi infecting a large number of plant species.
34 Depending on the *Fusarium* species, colonisation of the host plant is often accompanied by
35 production of various mycotoxins, including trichothecenes, which are of concern with
36 respect to food and feed safety (Kimura et al. 2007). According to their chemical structure,
37 trichothecenes are categorised into four different types, where type A and B trichothecenes
38 often occur in *Fusarium* infected cereals (Foroud and Eudes 2009). The type B trichothecene
39 deoxynivalenol (DON) is regarded the most frequently detected mycotoxin worldwide in
40 cereals and cereal products. Depending on cereal species, production stage and intended use
41 of the foodstuff, the European Union (EU) has set legal limits for DON varying between 200
42 and 1,750 $\mu\text{g kg}^{-1}$ (European Commission 2006). The EU has called for more data concerning
43 the occurrence, behaviour and toxicity of T-2- and HT-2-toxins and legal limits for these
44 toxins had been envisaged to be set by July 2008 (European Commission 2006). However,
45 these limits have presently not been established.
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58 Toxicity levels of T-2-toxin are approximately 10 times higher than those of DON (Ueno
59 1983). Accordingly, a relatively low temporary Tolerable Daily Intake (tTDI) for the sum of
60 T-2 and HT-2 of 0.06 $\mu\text{g kg}^{-1}$ body weight has been proposed by the Scientific Committee of
Food (European Commission 2001). It has been found that in oats frequency of occurrence

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3 and levels of T-2- and HT-2-toxins are considerably higher than e. g. in wheat, rye and soy
4 bean. However, in 2001 the data base was regarded as being too small to assess, if daily up-
5 take of the toxins with food was on average below the tTDI (JECFA 2001). In recent years
6 several authors have studied the occurrence of T-2- and HT-2-toxins in diverse cereals and
7 cereal products, e.g. in conventional and organic bread (Schollenberger et al. 2005), in diverse
8 grains and feedstuff (Schollenberger et al. 2006), in conventionally and organically grown
9 oats (Gottschalk et al. 2007; Edwards 2009b) and in barley (Edwards 2009a). It was also
10 assessed, whether toxin levels were reduced during processing of oats (e.g. de-hulling) and
11 malting and brewing of barley (Edwards et al. 2009). In Germany, there has been performed a
12 relatively large survey of various types of foods for levels of T-2- and HT-2-toxins (Curtui et
13 al. 2009). The outcome of the latter survey was that toxins were found at low concentrations
14 in a large percentage of cereal based products, where highest frequency of occurrence and
15 highest median values for T-2- and HT-2-toxins were obtained in oat based products. The
16 relatively low concentrations of the toxins lead the authors to conclude that for the average
17 consumers health risks would not be expected. On the other hand, it cannot be ruled out that
18 high intake consumers, especially children, are at a health risk due to elevated levels of T-2-
19 and HT-2-toxins in oat based products. For this reason the current work examines two aspects
20 concerning levels of T-2 and HT-2 toxins in oats *i*) influence of different cultivars and
21 different growing areas within Germany and *ii*) influence of a range of processing steps
22 during the production of oat foodstuffs.
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41 **Material and methods**

42 *Sample collection, preparation and further treatment*

43 The oat samples from cultivar studies in 2007 were provided from the German Federal Plant
44 Variety Office (BSA) in Hannover, Germany. The four oat cultivars Aragon, Dominik, Ivory
45 and Pergamon were grown on ten different sites, distributed relatively evenly in Germany. On
46 every site, plants were treated analogous to normal agricultural practise with several
47 applications of fungicides. Plant treatments between sites were not uniform and differed due
48 to local conditions such as infection status and weather patterns (2010 personal
49 communication D. Rentel, BSA, unreferenced, see 'Acknowledgements'). At least 2 kg of the
50 harvested raw material was stored at room temperature in the dark until grinding an aliquot of
51 300 g per sample in a mill with 500 µm sieve pore size (Retsch, Haan, Germany). The ground
52 raw oat samples (n=40) were screened using direct competitive enzyme-linked
53 immunosorbent assays (ELISA) for simultaneous detection of T-2/HT-2-toxins according to
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3 Hocke (2008). Out of these samples, we chose eight samples with levels higher than 100 μg
4 kg^{-1} T-2/HT-2 for the further experiments.
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9 Approximately 1 kg each of the eight chosen raw oats samples were cleaned using a
10 laboratory scale grain cleaner (Labofix, Schmidt-Seeger, Beilngries, Germany), which
11 separates foreign material from the oat grains. Half of the cleaned oats were de-hulled with an
12 impact de-huller operating with compressed air (Figure 1). Approximately 0.5 kg of each,
13 cleaned and de-hulled oats, were ground separately as described above. The ground samples
14 were stored in glass bottles at room temperature in the dark until analysis and further usage
15 for processing experiments.
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23 For determination of T-2-/HT-2-toxin levels in the eight raw oats samples, the cleaned and
24 de-hulled oat samples as well as in the processed material we employed an LC-MS method.
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27 28 ***Preparing “porridge” from oat meal***

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30 “Porridge” was prepared by adding 20 g whole oat meal, obtained from cleaned oats, to
31 100 mL tap water. The mixture was stirred thoroughly and the porridge was heated (30 °C to
32 96 °C in 22 min, 1 min at 96 °C) in a Micro Visco-Amylo-Graph[®] MVAG (Brabender,
33 Duisburg, Germany), which ensures good mixing of the sample without sedimentation of
34 particles and steam loss. The preparations were done with a controlled temperature cycle of
35 heating and holding, using fixed shear conditions. This ensures highly reproducible
36 gelatinization and pasting profiles of the oat porridge. After this a freeze-drying procedure of
37 the porridge followed. The porridge preparation from each sample was performed in three
38 replicates, all of which were subsequently analysed for T2- and HT-2-toxins with LC-MS.
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47 48 ***Bread manufacturing***

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50 In order to obtain bread contaminated with T-2- and HT-2-toxins, wheat flour and whole oat
51 meal (obtained from cleaned oats) with known levels of T-2- and HT-2-toxins was used. The
52 bread recipe contained a mixture of 80% wheat flour and 20% whole oat meal. The wheat
53 flour quality (type 550) was characterised using various ICC-Standard methods. The flour had
54 14% water content (ICC 110/1), 385 s falling number (ICC 107/1), 11.6% protein content
55 (ICC 105/1), 27.4% gluten content (ICC 137/1), 36 mL sedimentation value (ICC 116/1),
56 57.8% water absorption (ICC 115/1) and 0.59% ash content (ICC 104/1). Descriptions of the
57 ICC methods are available in the internet at <http://www.icc.or.at/methods3.php>.
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3 The bread dough was mixed using 800 g wheat flour, 200 g whole oat meal, 50 g fresh
4 baker's yeast, 15 g pure peanut oil (partly hydrogenated), 15 g white refined sugar, 20 g table
5 salt and 580 g water. Manufacturing conditions were in principle identical with industrial
6 bread making processes. Details of the conditions are listed in Table 1. For each sample bread
7 manufacturing was replicated three times.
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13 ***Bread and porridge sample preparation***

14 Bread samples were ripped into small pieces and air dried for two days at room temperature.
15 Dried bread and freeze-dried porridge samples were ground in a mill (500 µm sieve pore size,
16 Retsch, Haan, Germany). Ground material was stored in glass bottles in the dark. Water
17 content of all materials were analysed with a moisture analyzer (Sartorius AG, Göttingen,
18 Germany).
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26 ***Chemicals***

27 Methanol (LC-MS grade) was purchased from Roth (Karlsruhe, Germany) and acetonitrile
28 (HPLC grade) was obtained from Baker (Deventer, The Netherlands). Formic acid p.a. was
29 from Merck (Darmstadt, Germany) and the standard solutions of T-2- and HT-2-toxin, both in
30 acetonitrile, were purchased from Biopure (Tulln, Austria).
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37 ***Analytical methods***

38 ***Enzyme-linked immunosorbent assay (ELISA)***

39 Briefly, ground oat material (5 g per extraction) was extracted with 70% methanol in water
40 (v/v). After centrifugation the supernatant was cleaned by liquid-liquid extraction with ethyl
41 acetate. Extracts were assayed in quadruplicate for concentration of T-2- and HT-2-toxins
42 using microtitre plates. The limit of quantification for T-2- and HT-2-toxins was 0.5 µg kg⁻¹
43 and recoveries were greater than 80%. In interlaboratory studies a good reproducibility of the
44 method was obtained: in 11 samples the RSD_R values were between 9 % and 31 % and the
45 mean RSD_R was 17% (Hocke 2008). Using the described ELISA method and a GC based
46 method for detection of T-2- and HT-2-toxins on identical samples, there was a high
47 correlation in the results ($r^2 = 0.96$), (Majerus et al. 2008).
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56 ***Liquid Chromatography Mass Spectrometry (LC-MS) -method***

57 ***Pressurized liquid extraction (PLE) and extract treatment.*** Ground material (10 g) was mixed
58 with 4 g diatomaceous earth (Varian, Darmstadt, Germany) for keeping the sample porous.
59 This mixture was placed on top of a layer of 2 g activated charcoal (Merck) in a 33 mL
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3 stainless steel cell (Dionex, Idstein, Germany). Extraction was done using an Accelerated
4 Solvent Extractor (ASE 300) instrument (Dionex) with 20% water in acetonitrile (v/v) as
5 solvent under the following conditions: preheat 5 min, static 5 min, flush 130%, purge 80 s,
6 cycles 5, pressure 15000 kPa, temperature 85 °C. The extract volume was adjusted to 100 mL
7 with 20% water in acetonitrile (v/v) in a volumetric flask. A 10 mL aliquot of the extract was
8 evaporated to dryness and subsequently redissolved in 0.3 mL methanol and 1.2 mL Milli-
9 Q[®]-water (Millipore, Schwalbach, Germany). After filtration through a 0.2 µm disposable
10 syringe filter (Macherey-Nagel, Düren, Germany) extracts were transferred to vials and stored
11 if necessary at minus 20 °C prior to analysis.

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14 *Liquid Chromatography-System.* Separations were performed using a LC- prominence system
15 (Shimadzu, Duisburg, Germany) equipped with a Luna[®] phenyl-hexyl-column (150 x
16 2.0 mm I.D. particle size 5 µm) and a security guard (Phenomenex, Eschborn, Germany) at
17 40 °C. The flow rate was 0.4 mL per minute and a gradient was mixed using Milli-Q[®]-water
18 with 1% (v/v) formic acid as solvent A and methanol as solvent B applying the following
19 gradient programme: start 10% solvent B, 10-40% solvent B (1 min), 40% solvent B (3 min),
20 40-80% solvent B (6 min), 80% solvent B (4 min), 80-10% solvent B (0.5 min), 10% solvent
21 B (2.5 min).

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24 *Mass spectrometry.* The linear ion trap triple quadrupole mass spectrometer (4000QTrap,
25 Applied Biosystems, Darmstadt, Germany) with a TurboV[®]-Source was operated in ESI(+)-
26 multi reaction mode (MRM, Table 2) with the following general settings: Curtain gas 28 psi,
27 CAD gas medium, IS 5500 V, temperature 315 °C, Gas1 50 psi, Gas2 75 psi.

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30 Quantification was carried out with a matrix matched calibration curve. For this purpose
31 matrix standards were prepared by extracting a blank sample (each for raw oats, cleaned oats
32 bread and porridge respectively) like any other sample until evaporation to dryness (see step
33 PLE). Dried samples were dissolved in 1.2 mL water and 0.3 mL standard T-2-/HT-2 solution
34 in methanol. Calibration-matrix-standards had concentrations between $1 \cdot 10^{-6}$ and $7 \cdot 10^{-4}$ g L⁻¹
35 T-2- and HT-2-toxin in 20% methanol/water (v/v), respectively. Detection limits (Signal to
36 noise ratio ≥ 3) were between $1 \mu\text{g kg}^{-1}$ and $3 \mu\text{g kg}^{-1}$ for T-2-toxin and between $3 \mu\text{g kg}^{-1}$
37 and $5 \mu\text{g kg}^{-1}$ for HT-2-toxin, depending on the matrix.

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40 *Matrix effects and recovery.* To determine the matrix effects the response factors (RF) of the
41 peak areas were calculated as a function of the analyte concentration added with the matrix
42 matched calibration standards. The results for the RFs of T-2 and HT-2 in raw oats, porridge
43 and bread are shown in Figure 2. The response factors within a specific matrix were very
44 constant down to a concentration of $30 \mu\text{g kg}^{-1}$. At toxin concentrations lower $30 \mu\text{g kg}^{-1}$
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3 RFs became more variable. Concentration dependent RFs of T-2- and HT-2-toxins were
4 different in raw oats, bread and porridge samples, respectively. RFs were lowest and little
5 variable for the heated and highly stirred porridge samples, indicating a high, but
6 homogeneous content of interfering organic matrix. The RF values for bread samples were
7 generally factor four higher than in porridge. In bread RF values were very even between 30
8 and 600 $\mu\text{g kg}^{-1}$ T2-/HT-2-toxins and increased steadily, when toxin concentrations were
9 lower 30 $\mu\text{g kg}^{-1}$. The raw oat samples exhibited highest and slightly more variable RFs. For
10 both, T2-toxin and HT-2-toxin a similar dependency of the RFs from the sample matrix was
11 found.
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14 In order to assess recovery of the toxins in the assay system, spiking experiments were done
15 by adding 1, 2 or 5 μg T-2 and HT-2 to 10 g of a blank sample in three replications. After an
16 incubation time of two hours, the samples were processed as described previously. Recovery
17 data for T-2-toxin were 72% in cleaned oats, 88% in bread and 93% in porridge, respectively.
18 For the HT-2-toxin we obtained 100% recovery in cleaned oats, 85% in bread and 102% in
19 porridge.
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32 **Results and discussion**

33 On ten different agricultural sites, distributed relatively evenly in Germany the four oat
34 cultivars Aragon, Dominik, Ivory and Pergamon had in sum T-2/HT-2 levels ranging from 9
35 to 623 $\mu\text{g kg}^{-1}$, as determined with ELISA (Table 3). Over all cultivars and all growing sites
36 the median was 48 $\mu\text{g kg}^{-1}$, the mean was 102 $\mu\text{g kg}^{-1}$ and the 90th percentile was 191 $\mu\text{g kg}^{-1}$
37 T-2-/HT-2-toxin. In comparison, oats from various regions in the UK showed nearly five
38 times higher values, i. e. for these oats the median and the mean values of the sum of T-2- and
39 HT-2-toxins were 213 $\mu\text{g kg}^{-1}$ and 570 $\mu\text{g kg}^{-1}$, respectively (Edwards 2009b). Our data are
40 more in accordance with results obtained for oats sampled in Germany: e.g. oats from south-
41 west Germany (n=17), collected in the years 2000 and 2001. They had maximum levels of
42 approximately 800 $\mu\text{g kg}^{-1}$ with a mean of 255 $\mu\text{g kg}^{-1}$ T-2/HT-2 (Schollenberger et al. 2006).
43 Similarly, oats including hulls (n=19) and de-hulled oats (n=21) contained in the mean 118
44 and 6.9 $\mu\text{g kg}^{-1}$ T-2/HT-2, respectively (Curtui et al. 2009). Taken together, these findings are
45 in support of the notion that levels of T-2/HT-2 are elevated in the northern European
46 countries and in the UK (Langseth and Rundberget 1999; Scudamore et al. 2007; Pettersson et
47 al. 2008) compared to Germany in the more central area of Europe.
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3 In this study the T-2/HT-2 levels at one agricultural site were approximately six times higher
4 compared to the other sites (Table 3), showing that higher contents of T-2/HT-2 in German
5 oats can occur. This result might be due to local conditions at this agricultural site. Anyway,
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7 80% of all samples in this study here were below $100 \mu\text{g kg}^{-1}$, whereas only 10% showed
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9 higher T-2/HT-2-toxin levels with more than $300 \mu\text{g kg}^{-1}$.
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14 Compared to the mean of all cultivars on an individual site, the cultivars Pergamon and Ivory
15 had lower T-2/HT-2 levels for 9 and for 8 out of 10 sites, respectively (Table 3). This result
16 suggests that these cultivars might be less sensitive to infection with *Fusarium*. By contrast,
17 for the cultivars Aragon and Dominik only 6 (Aragon) or 2 (Dominik) sites had lower values
18 than the mean. The result that Pergamon and Ivory contained decreased levels of T-2/HT-2-
19 toxins compared to the average of the cultivars appears promising with respect to the aim of
20 lowering the levels of these toxins in foodstuff. Clearly, more research in this direction is
21 necessary, especially as there are only a limited number of publications addressing the issue
22 of T-2/HT-2 levels in different oat cultivars. In a study on twelve raw oat samples from the
23 UK, Sweden and Finland Scudamore et al. (2007) found in every sample T-2/HT-2-toxins,
24 where concentrations ranged from 143 up to approximately $3500 \mu\text{g kg}^{-1}$. However, they did
25 not match their finding with different cultivars. In another study on oats in the United
26 Kingdom, the variety Gerald cited as the most popular oat variety in the UK had the highest
27 T-2/HT-2 concentration (Edwards 2007). In summary, the influence of the oat variety on the
28 T-2/HT-2 level appears to be noticeable and should be further investigated.
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42 Eight of the raw oat samples were chosen for various cleaning and food processing
43 procedures. In the course of these experiments all analyses of T-2/HT-2-toxins were carried
44 out using an LC-MS-method, yielding the sum of, and most advantageously, also individual
45 levels of T-2- and HT-2-toxins. In 7 of the 8 raw oat samples HT-2 levels were higher than T-
46 2 levels, where the ratio between HT-2 and T-2 levels ranged between 2.1 and 8.7 (Table 4).
47 Similar ratios in naturally contaminated oats were found in other surveys also involving oats
48 from Europe (Scudamore et al. 2007).
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56 Cleaning the raw oat material using a laboratory grain cleaner led to varying effects on levels
57 of T-2/HT-2-toxins in the grain. Whereas a moderate reduction of toxin levels in the range of
58 10 to 15% was achieved for highly contaminated samples 5, 6 and 8, no reduction of toxin
59 levels was observed for samples 4 and 7. In contrast, samples 2 and 3 showed a 100% and
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3 sample 1 a 69% reduction of toxin concentrations after cleaning. The ratio between both
4 mycotoxins in the cleaned oats was nearly the same as in the raw oats. Overall, there was no
5 consistent effect of cleaning using the “Labofix” grain cleaner on levels of T-2/HT-2-toxins in
6 the eight samples.
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11 A further step in processing of oats is the de-hulling procedure. The glumes (husks) have to
12 be separated from the oat grain before preparing e.g. oat-flakes and other products for direct
13 human consumption. The T-2/HT-2-toxin levels of de-hulled oats are also presented in Table
14 4. De-hulling of oats led to a T-2/HT-2-reduction of 98% in the mean, where reduction varied
15 between 93.8 and 100%. These are results similar to those in oats from northern Europe
16 (Scudamore et al. 2007) and Canada (Clear et al. 1997). The major reduction of T-2/HT-2-
17 toxins obtained by de-hulling clarify, why the consumer products from retail markets in
18 Germany had very little T-2-/HT-2 contents although higher concentration in raw oats were
19 found (Curtui et al. 2009). An interesting result from this work here is that after de-hulling,
20 exclusively T-2 toxin was found at low levels in kernels, indicating that HT-2 toxin is more
21 strictly confined to oat hulls (Table 4).
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33 The further investigations on cooked porridge and bread were done using cleaned raw oats as
34 starting materials for cooking and baking, because these oats had higher toxin levels than the
35 de-hulled oat kernels. Utilisation of de-hulled kernels would very probably have resulted in T-
36 2/HT-2-toxin levels close to the limit of detection (LOD) in the final products, which has to
37 be avoided from the analytical point of view.
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43 The cooking of the ground cleaned raw oats with water was performed in an increasing
44 temperature gradient of 3°C per min from 30°C to 96°C and a holding time of 1 min at 96°C.
45 In the resulting “porridge” the mean T-2/HT-2-toxin levels varied from minus 40 to plus 46%
46 compared to the theoretical value of T-2/HT-2-toxins that would have been expected in the
47 various samples without any T-2/HT-2-toxin alteration (Figure 3). At the present stage of
48 experimentation we would not suggest that a significant difference between cooking treatment
49 and T-2/HT-2-toxin level exists, because standard deviations in the cooked samples were
50 relatively large. Analysis of matrix effects and recovery indicate that the analytical method is
51 not the major cause of the observed variations (see Material and Methods section), leading us
52 to conclude that the variations are due to the porridge production procedure. To our
53 knowledge this is the first time T-2/HT-2-toxin levels were examined comparing raw and
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3 cooked oats. Despite the variations, the absolute T-2/HT-2-toxin levels were relatively stable
4 during short time cooking. This is especially relevant for infant and baby food processing, as
5 they are the consumers with a relative high consumption of cooked oats.
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10 Observation of the T-2/HT-2-toxin levels during baking experiments was also carried out
11 using the ground cleaned raw oats. The four highest contaminated oat meals were baked to
12 bread in triplicate each. After baking, the resulting mean T-2/HT-2-concentration in the bread
13 was minus 23% compared to the theoretical value of T-2/HT-2 levels without baking (Table
14 4). Variations ranged from plus 12 to minus 44%. The significance of the result is that only a
15 moderate reduction of toxins levels can be achieved during the baking process. One
16 explanation is that T-2-/HT-2-toxins are relatively stable at elevated temperatures. As with
17 preparation of porridge, the variations of the toxin levels are probably due to the bread
18 making and baking processes, since parameters of the analytical method had no major
19 influence (see Material and Method section). Both, preparation of porridge and bread making
20 need to be further evaluated with respect to behaviour of T-2- and HT-2 levels under
21 controlled conditions.
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33 Our results illustrate that concentrations of T-2-and HT-2-toxins in oats from Germany can
34 vary considerably, depending on variety and also location. Whereas the efficacy of cleaning
35 was limited, de-hulling had a major reduction effect on T-2- and HT-2-toxin levels in oats. At
36 the present stage of experimentation, results of food processing experiments indicated that
37 cooking of porridge and bread making had little effect on T-2/HT-2-toxin levels.
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46 making available the oat samples and for providing information concerning the cultivation
47 conditions of the oat varieties that are contained in internal documents not accessible to
48 scholars. The screening work using the T-2/HT-2 antibodies was financially supported by the
49 BMELV (Project No. 05HS001).
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5 Figure 1. Schematic diagram of the procedures and the equipments used for preparing oat
6 based whole meal, porridge and bread.
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10 Figure 2. Response factors for T-2- and HT-2-toxins obtained from matrix matched
11 calibration standards. Triangles represent T-2- and rhombuses HT-2-toxin in raw oats, bread
12 and porridge.
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17 Figure 3. T-2/HT-2 toxins in cooked porridge samples. Porridge was prepared from five
18 different oat samples (n=3). Mean values and standard deviations as well as the theoretical
19 content that would be expected without cooking are presented.
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24 Figure 4. T-2/HT-2 toxins in bread. Bread was prepared from four different oat samples
25 (n=3). Mean values and standard deviations as well as the theoretical content that would be
26 expected without baking are presented.
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Table 1. Terms and conditions of bread manufacturing process.

Process parameter	condition	special equipment
kneading	1 min slow / 5 min fast	DIOSNA spiralkneader, type SP 8
dough temperature	26 °C	
dough rest	10 min	
dough per bread	dough weight/2 = ~0.8 kg	
intermediate proofing	15 min	
forming	manuell	
final proofing		MANZ proofer, type G 86 W
- relative humidity	80%	
- temperature	32 °C	
- proofing time	35 min	
baking conditions		DAUB Backmeister, type BTO
- steam jet	10 s	21613-S
- baking temperature	220 °C	
- baking time	40 min	

Table 2. MRM transitions and conditions.

Analyte	Q1 mass [amu]	Q3 mass [amu]	declustering potential [V]	entrance potential [V]	collision energy [V]	cell exit potential [V]
T-2	467	305	40	10	17	19
	467	245	45	10	17	19
HT-2	425	263	50	10	12	15
	425	105	50	10	12	15

Table 3. Levels of the sum of T-2/HT-2 ($\mu\text{g kg}^{-1}$) in four oat cultivars from ten agricultural sites that are distributed evenly in Germany and mean levels of T-2/HT-2 ($\mu\text{g kg}^{-1}$) of all four cultivars at one site. Data acquired with ELISA.

Site number	Aragon	Dominik	Ivory	Pergamon	mean at one site
1	67	40 ^a	33 ^a	36 ^a	44
2	100 ^{a,b}	120 ^b	95 ^a	163	120
3	28 ^a	87	9 ^a	20 ^a	36
4	42 ^a	42 ^a	129	28 ^a	94
5	61	50	34 ^a	26 ^a	43
6	104 ^b	118 ^b	39 ^a	48 ^a	77
7	15 ^a	69	19 ^a	20 ^a	31
8	26 ^a	54	27 ^a	31 ^a	34
9	623 ^b	570 ^b	584 ^b	322 ^{a,b}	525
10	14 ^a	20	14 ^a	10 ^a	15

Notes: ^a value lower than the mean of all cultivars at the same agricultural site. ^b samples chosen for further experiments involving cleaning and processing procedures.

Table 4. Sum and individual levels of T-2- and HT-2-toxin ($\mu\text{g kg}^{-1}$) in raw, cleaned and de-hulled oats and ratio of both toxins in the samples. Data acquired with LC-MS.

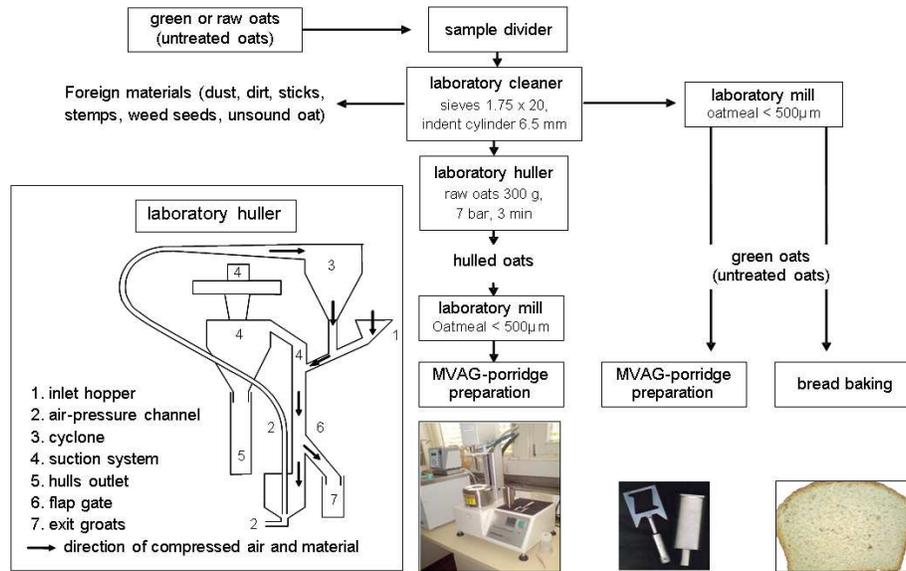
Sample	raw oats				cleaned oats				de-hulled oats			
	sum (T-2+HT-2)	T-2	HT-2	ratio HT-2/T-2	sum (T-2+HT-2)	T-2	HT-2	ratio HT-2/T-2	sum (T-2+HT-2)	T-2	HT-2	ratio HT-2/T-2
1	104	23	81	3.5	36	*	36	-	-	*	*	-
2	171	40	130	3.3	-	*	*	-	-	*	*	-
3	14	14	*	-	-	*	*	-	-	*	*	-
4	155	30	125	4.2	156	30	126	4.2	-	*	*	-
5	670	214	456	2.1	569	150	419	2.8	13	13	*	-
6	845	87	758	8.7	765	85	680	8.0	-	*	*	-
7	476	101	375	3.7	486	85	401	4.7	29	29	*	-
8	584	156	428	2.7	436	96	340	3.5	15	15	*	-

Notes: mean data of 3 replicates, determined with LC-MS. For T-2 the Limit of Quantification ($S/N \geq 6$) was 6 and for HT-2 the Limit of Quantification ($S/N \geq 6$) was 15. *indicates that values were below the LOQ.

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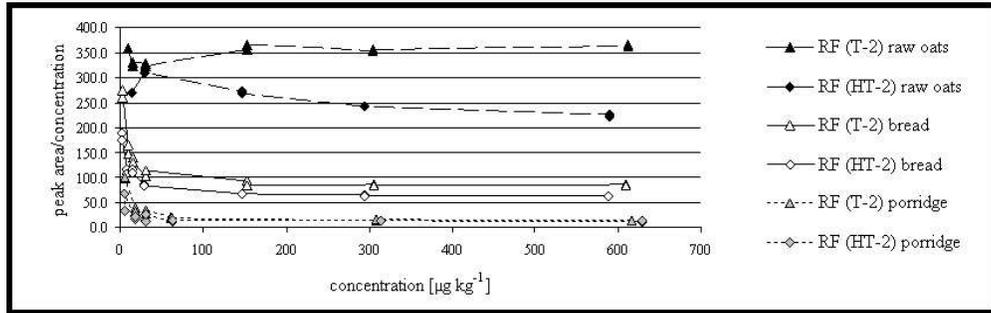
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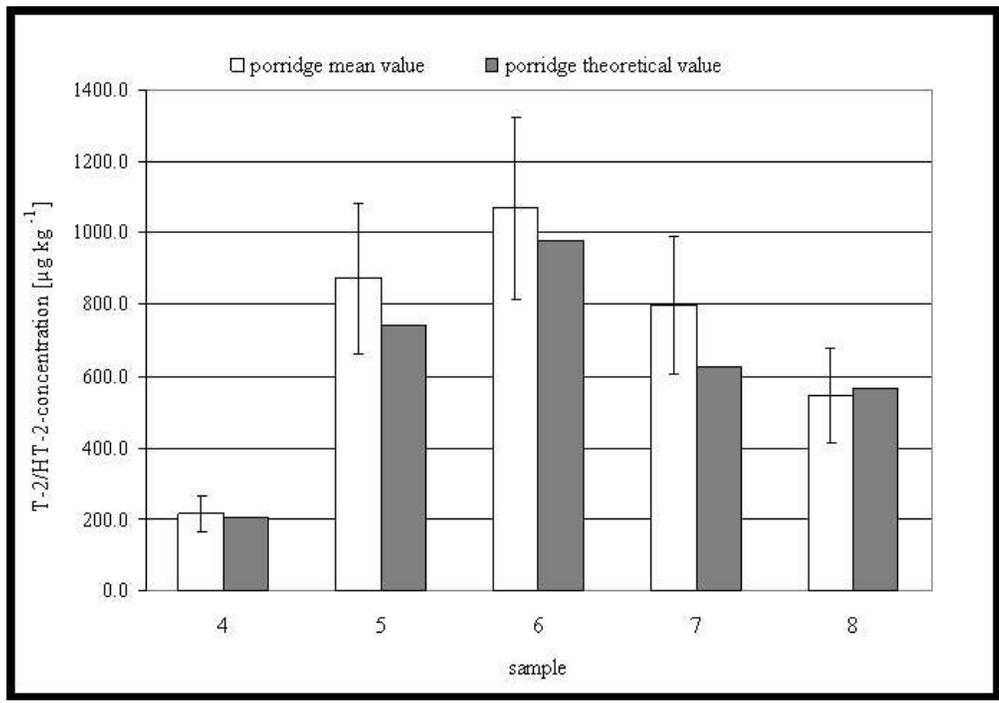
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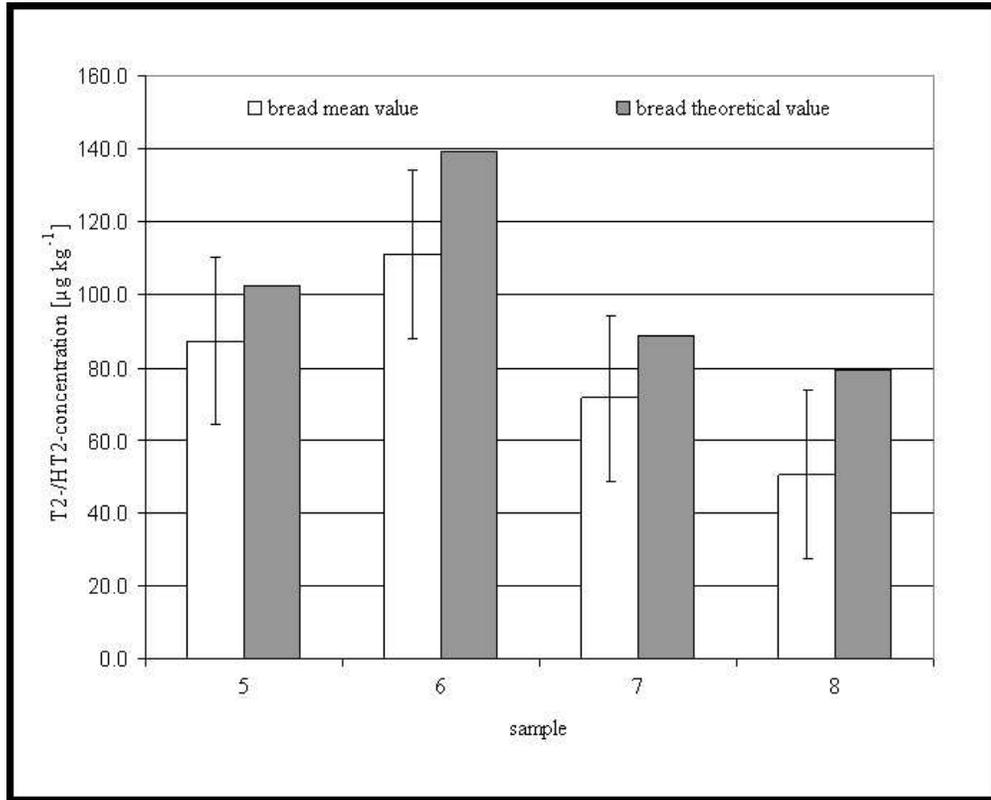
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