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Impact of *Fusarium* spp. infection of bread wheat (*Triticum aestivum* L.) on composition and quality of flour in association with EU maximum level for deoxynivalenol

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Summary

Contamination of grain with mycotoxins such as deoxynivalenol (DON) is the major threat after *Fusarium* head blight (FHB) infection of wheat; however technological quality can also be impaired. The European Union has established maximum levels (ML) of DON for wheat grain and foodstuffs. The composition (starch, gluten proteins) and quality (protein content, sedimentation value, wet gluten, water absorption, mixing properties of dough, baking volume) of 72 flour Type 550 samples from two years either fulfilling or exceeding ML of 0.75 mg kg^{-1} were investigated. DON content of flours ranged widely from below limit of quantification to 11.84 mg kg^{-1}. Aside from a slight loss of loaf shape in flours highly exceeding ML, no negative effect on composition and quality of flour was observed in flours exceeding ML compared to those fulfilling ML. A significant decrease in total glutenin and LMW-GS content did not correlate with any quality trait. Hence, if flours fulfill ML for DON, reduced technological quality due to FHB is not significant.

Introduction

Wheat is one of the most important staple foods worldwide. Together with rice and maize, it provides about 60 % of the world's food energy intake (FAO, 2014). In Germany, winter wheat covers about 44 % of the total cereal production area (STATISTISCHES BUNDESAMT, 2013), while each citizen consumes about 71 kg wheat flour (STATISTISCHES BUNDESAMT, 2013) as well as 85 kg bread and bakery products per year (BELV, 2009). Due to its great importance in the human diet, especially as bread wheat, the production of wheat with a minimum content of contaminants is of major interest. *Fusarium* head blight (FHB) of wheat is a worldwide-occurring disease with tremendous economic importance. During the 1990s, FHB of wheat and barley caused about $3 bn losses due to reduced yield, grain and seed quality in the United States (WINDELS, 2000). *Fusarium graminearum* is generally described as the predominant specie causing FHB (OSBORNE and STEIN, 2007). In Europe, deoxynivalenol (DON), its acetylated derivates (3-ADON, 15-ADON), and zearalenon (ZEN) are the most frequently detected mycotoxins in wheat and wheat products (BOTTALICO and PERRONE, 2002). The toxicological effects of these mycotoxins on mammals range from anorexia to impaired reproduction (PESTKA, 2010; ZINEDINE et al., 2007). Therefore the European Union has established maximum levels (ML) of DON and ZEN for cereals and cereal products, including foodstuffs produced from wheat (Commission Regulation (EC) No. 1881/2006). Accordingly, unprocessed wheat, cereal flour, bread and wheat-based foods for infants and young children must not contain more than 1250, 750, 500, and 200 as well as 100, 75, 50, and 20 µg kg^{-1} for DON and ZEN, respectively.

Aside from the toxicological risk resulting from mycotoxin contamination of wheat, wheat quality may be impaired since FHB infection may influence grain components, such as starch and proteins (SIUDA et al., 2010) and subsequently end-use quality traits, as pasting properties (WANG et al., 2005a) and baking performance (LANCOVA et al., 2008). WANG et al. (2005b) found the composition of gluten proteins, which contribute considerable to the baking properties of wheat, altered as a result of FHB. A distinct reduction in the content of both total glutenin and high-molecular-weight glutenin subunits was detected in wheat after serious artificial infection. As a result, the dough quality was impaired and consequently, an unsatisfactory bread quality was observed.

Biochemical changes in grain composition and subsequent changes in wheat quality traits caused by FHB might result from both fungal enzymes and impaired synthesis of grain components. It is assumed that *Fusarium* ssp. secrete enzymes, such as carbohydrases and proteases, during the invasion of the kernel thus degrading starch, cell wall components, and gluten proteins (EGGERT et al., 2011; DEXTER and NOWICKI, 2003; PEKKARINEN et al., 2000). *Fusarium* infection might also lead to an incomplete accumulation of kernel constituents through the mechanical blocking of vascular bundles by fungal mycelium (GOSWAMI and KISTLER, 2004; KANG and BUCHENAUER, 2000; RIBICHICH et al., 2000) or thorough impaired synthesis of grain components due to the presence of mycotoxins (ERIKSEN and PETTERSSON, 2004; CASALE and HART, 1988).

This study investigates how wheat quality is affected if ML of DON for flour of 0.75 mg kg^{-1} is fulfilled or exceeded. Both flour composition and common quality parameters of wheat, including ultimate criterion of baking volume, are investigated. ML for DON was used because ZEN content of flour samples was insignificant. The study uses flour Type 550 which is the most processed bread flour in Germany. Flour was milled from naturally *Fusarium* infected grain. Earlier studies, investigating the relationship of *Fusarium* infection and wheat quality, mainly investigated only severely artificially infected samples (GARTNER et al., 2008; PRANGE et al., 2005) or unprocessed grain (LANCOVA et al., 2008; WANG et al., 2005a, b). Others compared several kernel classes ranging from lightly to severely *Fusarium* damaged grain (SIUDA et al., 2010; BOYACIOGLU and HETTIARACHCHY, 1995; BECHTEL et al., 1985). The often cited study of NIGHTINGALE et al., 1999 demonstrated impaired wheat quality in in *Fusarium* damaged wheat that comprised 88.0-113.0 mg kg^{-1} DON. Severely *Fusarium* damaged kernels and highly DON contaminated wheat and wheat products are suitable to demonstrate a potential *Fusarium* damage on grain but rather lack practical relevance since a grain lot is a mixture of all kernel classes, removal of severely and moderately infected kernels by cleaning procedures is possible to a certain degree (SEITZ et al., 1986), and maximum level of DON is a legal criterion of exclusion. Therefore earlier studies might have overestimated the impact of FHB on baking quality wheat within the legal range of DON.

Material and methods

Plant material

Flour Type 550 were gained from *Fusarium* infected wheat grain harvested in a field experiment carried out from 2007 and 2009 at two locations (Torland, Gladebeck) near Goettingen, in the south of Lower Saxony, Germany. Natural variation of DON within the samples resulted from the experimental factors including pre-crop (wheat, maize, sugar beet), cultivar (highly/hardly susceptible against

FHB), and fungicide treatment (strobilurin, triazole, chlorathalonil applied during tillering). A complete description of the field experiment is given in KREUZBERGER (2011). Two common German bread wheat cultivars were cultivated, alike in quality parameters (according to Federal Office of Cultivar Variety) but differing in susceptibility against FHB; Ritmo shows high susceptibility against FHB (FHB+), Centrum is hardly susceptible against FHB (FHB-). Grain was harvested by combine harvester (Farmliner, Deutz-Fahr, Cologne, Germany) at a grain moisture content of 14.5-16%.

Post-harvest treatment

Thirty-six bulk samples of always six kg were created per year within each factor treatment of the trial. Samples were cleaned once with the standard settings of A/S Rationel Kornservice, Esbjerk, Denmark. After one month of storage two kg of each bulk sample were tempered for 12 h at ambient temperature to gain a moisture content of 15.5%. After that grain was milled into flour with an extraction rate of 75% and an average mineral content of 0.55% in dry matter, also known as flour Type 550 (Buhler laboratory mill type MLU 202, Uzwil, Switzerland; Quadrumat Senior 220/380, Brabender, Duisburg, Germany). Mineral content of flour was determined slightly modified according to ICC STANDARD NO 104/1. Five g of flour were dried until constant weight and incinerated in a muffle furnace for 4 h at 900 °C. Flour samples were kept in air-tight bottles (PE) at 4-8 °C in the dark until analysis.

Quantification of deoxynivalenol

DON determination was performed by LC-MS/MS. Five g of flour were extracted with 50 ml acetonitrile-water (84:16, v/v). The extraction solvent was replaced with methanol-isopropanol-water (80:5:15, v/v/v) during the so-called acetonitril crisis in 2008/2009. The extracts were cleared, defatted, dried, dissolved in methanol-water (1:1, v/v) and filtered as previously described (ADEJUMO et al., 2007) and injected into a C_{18} column (Kinetex, 50 x 4.6 mm i.d., 2.6 µm; Phenomenex, CA, USA), equipped with a pre-column containing the same C_{18} material. A gradient of solvent A (5 mM acetic acid in water containing 5% acetonitrile) and solvent B (5 mM acetic acid in methanol) was used as the mobile phase. DON was detected by tandem mass spectrometry (Varian 1200L MS/MS system (Varian, Inc. CA, USA) equipped with a triple quadrupole mass spectrometer, two ProStar 210 liquid chromatographic pumps, a 410 autosampler, and a 500 MS Ion Trap mass spectrometer with ESI interface). The triple quadrupole settings (ADEJUMO et al., 2007) and mass transitions (KLOTZEL et al., 2006) were previously described. Calibration curves were prepared by spiking certified analytical standards into blanks of flour, which were then processed in the same way as the samples (matrix-matched standards). The limit of detection (LOD: 0.015 mg kg^{-1}) and quantification (LOQ: 0.10 mg kg^{-1}) were determined based on the signal-to-noise ratio. DON content was based on dry matter of flour.

Determination of flour composition

Starch

Starch content was determined according to ICC STANDARD NO. 123/1 and measured by polarimetric method (Polarimeter type V DrNa, Zeiss AG, Jena, Germany). Protein was precipitated with wolframatophosphoric acid. Starch content was calculated with the rotation angle of the filtrate at 589 nm. Each measurement was repeated twice.

Gluten proteins

Extraction of gliadins and glutenins from 100 mg of flour was conducted according to WIESER (2000). Before each extraction step, solvent and flour or sediment, respectively, were vortexed for two minutes. Albumins/globulins and gliadins were extracted at room temperature with magnet stirring (Variomag Multipoint 15, H+P Labortechnik AG, Oberschleissheim, Germany). Extraction of glutenins was performed for 20 min at 60 °C (Thermomixer comfort, Eppendorf AG, Hamburg, Germany) at 750 rpm. Suspensions were centrifuged (Centrifuge 5804R, Eppendorf, Hamburg, Germany) at 20 °C for 15 min at 7500 rpm. Each sample was extracted twice. Protein extracts were stored at -20 °C until measurement.

For quantification of protein fractions, gliadin and glutenin standards were prepared from commercially available gluten from wheat (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) as described by EGGERT et al. (2011). Protein content of isolated gliadin and glutenin was determined by C/N analyser (Vario MAX CN Elementar Analysensysteme GmbH, Hanau, Germany). Protein content of gliadin and glutenin standard were 93.41% and 100.00% of dry matter, respectively. Gliadin and glutenin standards were solved and determined accordingly WIESER (2000).

Quantification of gliadin and glutenin fractions followed with slight modification the description of WIESER et al. (1998) and EGGERT et al. (2010). For the RP-HPLC, a PerfectSil 300 C8 300 x 4.6 column (Machery-Nagel, Dueren, Germany) was used. Mobile phases were A = 0.1% trifluoroacetic acid (TFA) (Merck, Darmstadt, Germany) in H_2O (v/v) (Chromanorm, VWR, Fontenay-Sous-Bois, France) and B = 0,1% TFA in acetonitrile (v/v) (HiPerSolv, Chromanorm, VWR; Leuven, Belgium). All solvents were of HPLC grade. 100 µl of the aliquots of gliadin and glutenin were injected. The gradient conditions for separation and analysis of protein fractions were as followed: A, 100 - 76% in 0 - 5 min; 76 - 50% in 5 - 50 min; 50 - 10% in 50 - 54 min; 10 - 100%, 54 - 59 min. Flow rate was 1 ml min^{-1}; and the column temperature were set at 50 °C. Gliadin subfractions were separated into ω5-, ω1,2-, α-, γ-gliadins, whereas glutenins (GS) were separated into ωb-GS, HMW-GS (high molecular weight glutenins), and LMW-GS (low molecular weight glutenins). For the quantification of the protein fractions gliadin and glutenin standards were used. In order to eliminate the effect of starch accumulation and the adverse relationship with protein content in grain, quantity of gliadin, glutenin as well as subfractions were calculated as percentage of total protein in flour Type 550 (% protein). Total protein content was on average 11.7%, 10.2%, and 10.4% 2007, 2008, and 2009, respectively. HPLC system (MZ Analysentechnik, Mainz, Germany) consisted of autosampler (Jasco AS-2051 Plus Intelligent Sampler), oven (Jasco CO-2060 Plus Intelligent Column Thermostat), degaser (Jasco DG-2080-54 4-Line-Degaser), pump (Jasco PU-2080 Plus Intelligent HPLC Pump), gradient mixer (Jasco LG-2080-04 S), and detector (Jasco MD-2015 Plus Multiwavelength Detector). The chromatograms were analyzed by Jasco ChromPass Chromatography Data Systems Version 1.8.6.1.

Determination of quality parameters

Protein content

Nitrogen content was determined according to Dumas principle from 100 mg dried flour by C/N analyzer (Vario MAX CN Elementar Analysensysteme GmbH, Hanau, Germany). Protein content was calculated according to ICC STANDARD NO. 105/2 (N x 5.7).

Sedimentation value and wet gluten content

Sedimentation value and wet gluten content were determined according to ICC STANDARD NO. 151 and ICC STANDARD NO. 106/2, respectively.

Water absorption and mixing properties of dough

Water absorption and mixing properties (dough development time, dough stability, degree of softening) were obtained according to

modified ICC STANDARD NO. 115/1 using a valorigraph (Type FQA-205, Metefem, Budapest, Hungary) in 2007 and Farinograph (Brabender®, No. 901368, type 810 105 001, Duisburg, Germany) in 2009. Farinograms were analyzed with Brabender® Farinograph Version 4.0.3. Each measurement was repeated three times.

Microbaking test

Dough was prepared with 50 g flour (corrected to 14% moisture content), 1% sucrose, 1% fat, 1.5% salt, 5% fresh yeast, and 0.002% L-ascorbic acid based on flour weight. Water was added according to determined water absorption.

Dough was mixed for 2 min at 30 °C, proofed for 20 min at 30 °C in a water saturated atmosphere (Ecocell 55, MMM Medcenter Einrichtungen GmbH, Munich, Germany), separated into five dough balls of equal weight and relaxed for further 3 min. In order to remove air bubbles, dough balls were formed with a noodle machine (model Atlas 150, Marcato, Italy) and rolled into a log shape. Dough pieces were further proofed for 45 min. Baking was performed for 12 min at 240 °C (electric furnance, AEG Haustechnik, Nuernberg, Germany) according to KIEFFER et al. (1993). Baking volume was measured after loaves had cooled by rapeseed displacement.

Statistical analysis

Data were analyzed separately for 2007 and 2009 with SigmaPlot version 13.0, Systat Software GmbH, Erkrath, Germany. To describe factors influencing variation of DON content two-factorial analysis of variance (ANOVA) was conducted with raw data considering pre-crop (P) and cultivar (C) as main factors. Assumptions were normal distribution and homogenicity of variance checking residuals with q plot.

To investigate the difference in quality between flours fulfilling and exceeding ML flour Type 550 samples were divided into two groups independently of experimental factors: flours fulfilling ML of DON for flour ($\leq$0.75 mg kg^{-1}), and flours exceeding this level (>0.75 mg kg^{-1}). Statistical significance ($p<0.001$) between the two groups was calculated with two-tailed t test if raw data was normally distributed and exhibited equal variance. If these assumptions were not given Whitney-Rank Sum test was applied.

Results

DON content of flours ranged from 0.31 to 11.84 mg kg^{-1} and from <LOQ to 8.42 mg kg^{-1} in 2007 and 2009, respectively. Aside from year, most of the total variation in DON content of flour Type 550 basically resulted from the experimental factors pre-crop and cultivar (Fig. 1). The average DON level was significantly higher in 2007 than in 2009. In both years DON content was maximized after pre-crop maize in the strongly susceptible cultivar, followed by pre-crop wheat and sugar beet. Flour from the hardly susceptible cultivar contained in both years on average about 76% less DON than the strongly susceptible cultivar. However, there was interaction between pre-crop and cultivar.

The categorization of flour samples into the two groups of fulfilling or exceeding ML led to different group sizes within the two years. In 2007 only about 30% of the samples fulfilled ML, in 2009 over 50% of the samples fulfilled the limit (Fig. 2). DON content ranged from 0.31 to 0.75 mg kg^{-1}and from 0.79 to 11.84 mg kg^{-1} in the two groups in 2007. In 2009 DON content ranged from <LOQ (0.10) to 0.73 mg kg^{-1} and from 0.85 to 8.42 mg kg^{-1}.

Starch content of flours exceeding ML was about 2.5% increased compared to flours fulfilling ML in 2009 (Fig. 3). This effect was not visible in 2007. Other studies observed either no effect (WANG et al., 2005a) or found starch content decreased after severe *Fusarium* infection of grain (SIUDA et al., 2010; MATTHAUS et al., 2004; PAWELZIK et al., 1998).

Protein content did not differ between flours fulfilling or exceeding ML in both years (Fig. 3). Some studies also confirm that protein content was not affected by *Fusarium* infection (TERZI et al., 2007; PRANGE et al., 2005; DEXTER et al., 1996; WANG et al., 2005b; SEITZ et al., 1986). In contrast, other studies observed an increase (e.g. SIUDA et al., 2010; MATTHAUS et al., 2004; BOYACIOGLU and HETTIARACHCHY, 1995) or a decrease of protein content after severe *Fusarium* infection of grain (e.g. GARTNER et al., 2008; PRANGE et al., 2005; NIGHTINGALE et al., 1999).

Total gluten content did not differ between flours fulfilling or exceeding ML in both years (Fig. 3). However, gluten composition differed in both years (Fig. 4). Content of total gliadin (Fig. 4 A) and gliadin subfractions (ω5, ω1,2, α) showed no significant difference between the two groups in both years (not shown). That gliadins are not significantly influenced by FHB of wheat is supported by the

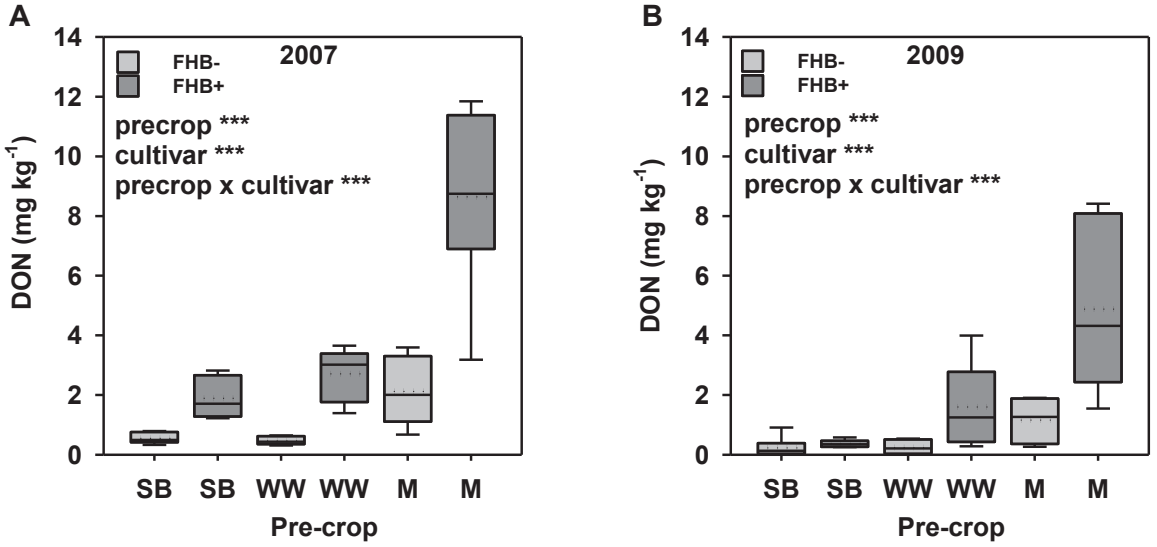

Fig. 1: Distribution of DON in flours Type 550 milled from wheat cultivated after pre-crop sugar beet (SB), winter wheat (WW), and maize (M) divided by cultivar hardly (FHB-) and highly (FHB+) susceptible against FHB in 2007 (A) and 2009 (B). Results of two-way ANOVA. Box plots show 25-75% percentile, median (solid line), and mean (dotted line).

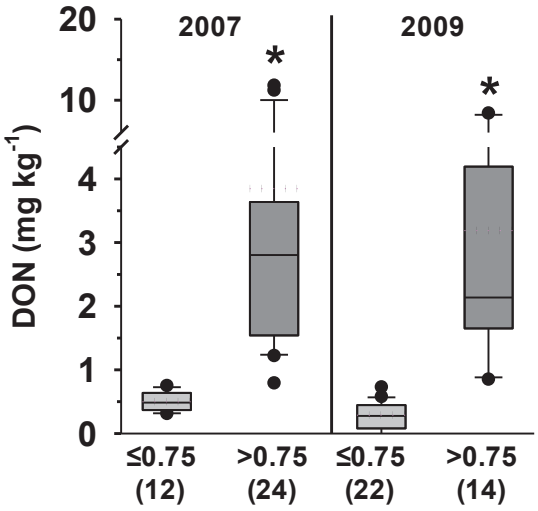

Fig. 2: DON contamination of flour Type 550 in 2007 and 2009 grouped into fulfilling (≤0.75) and exceeding (>0.75) EU maximum level of DON (mg kg^{-1}) for flour. In breaks – number of samples per group. Significant difference between groups within year is indicated through * ($p<0.001$). Box plots show 25-75% percentile, median (solid line), and mean (dotted line).

results of e.g. DEXTER et al. (1996) and PRANGE et al. (2005) where DON levels where similar or even exceeded DON of this study. On the contrary, WANG et al. (2005b) and EGGERT et al. (2010) observed a slight increase in total gliadin content and gliadin subfractions in severely *Fusarium* infected kernels of one wheat cultivar. This could only be observed for γ-gliadin in 2007 in flours exceeding ML. In 2009 this effect was not visible (not shown).

In contrast, in both years content of total glutenins was significantly reduced in flours exceeding ML compared to flours fulfilling ML (Fig. 4 B). The quantitative difference was about 20.6 and 10.8% in 2007 and 2009, respectively. This effect basically resulted from the lower LMW-GS content of these flours (Fig. 4 C). In 2007 and 2009 LMW-GS was about 22.4 and 11.7% lower in flours exceeding ML. Consequently gliadin-to-glutenin ratio was significantly increased in the samples exceeding ML (not shown). Content of HMW-GS was slightly decreased in flours exceeding ML in both years, however this difference was not significant. There was also no significant difference in ωb-GS (not shown). Other studies also detected a decrease of total glutenin however this was mainly due to decreased HMW-GS content (EGGERT et al., 2010; WANG et al., 2005b). They also observed an increase of LMW-GS which was contrary to the results of this study. On the contrary PRANGE et al. (2005) did not find an effect in flours that contained similar high DON levels as flours investigated in this study. WIESER and KIEFFER (2001) demonstrated that a changed gluten composition, more specifically a wider gliadin-to-glutenin or LMW-GS-to-HMW-GS, leads to impaired dough, gluten, and baking properties. Since in this study the quantitative change in gluten composition did not correlate with any of the quality parameters it can be regarded as insignificant.

Sedimentation value and water absorption of flours did not show any difference between the two groups in any year (Fig. 5 A, C). These results are confirmed by a few studies (e.g. SIUDA et al., 2010; GARTNER et al., 2008; DEXTER et al., 1996). On the other hand, several authors reported a decrease of sedimentation value (GARTNER et al., 2008; TERZI et al., 2007; WANG et al., 2005b; BOYACIOGLU and HETTIARACHCHY, 1995) and at least a slight increase of water absorption with increasing intensity of *Fusarium* infection (WANG et al., 2005b; PAWELZIK et al., 1998).

Wet gluten content was significantly increased about 14% in flours exceeding ML compared to flours fulfilling ML in 2007 (Fig. 5 B).

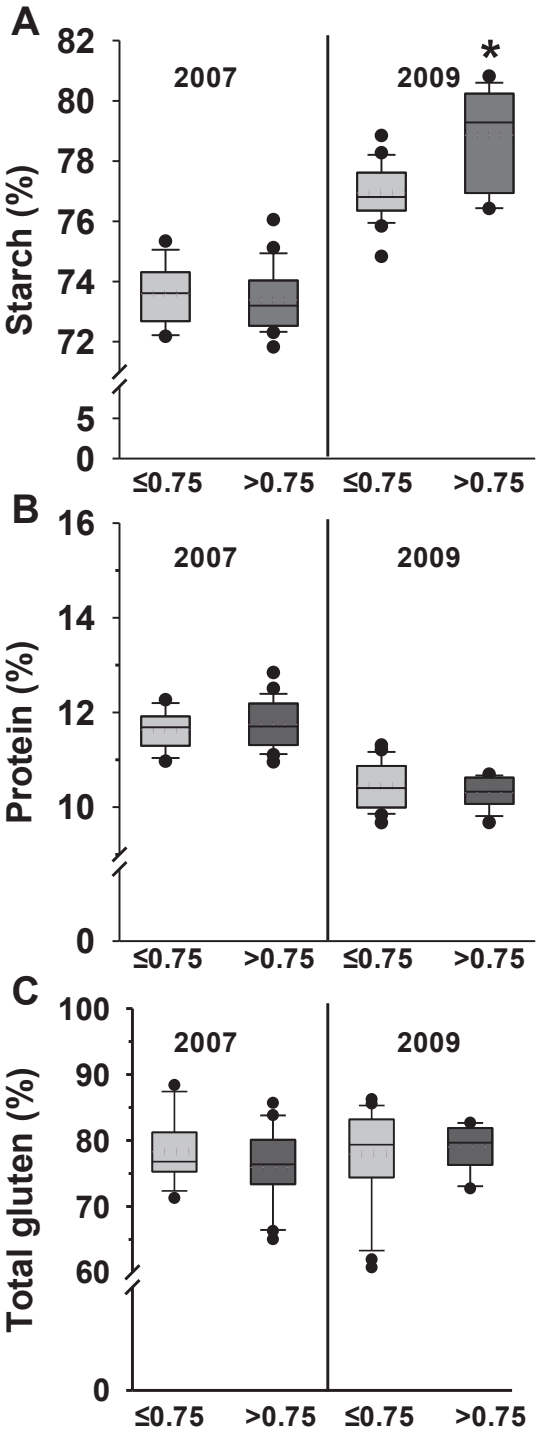

Fig. 3: Starch (A), protein (B), and total gluten (C) content of flour Type 550 samples in 2007 and 2009 grouped into fulfilling (≤0.75) and exceeding (>0.75) EU maximum level of DON (mg kg^{-1}) for flour. Significant difference between groups within year is indicated through * ($p<0.001$). Box plots show 25-75% percentile, median (solid line), and mean (dotted line).

This effect was former reported by SIUDA et al. (2010). In 2009 this effect was not visible which is supported by the findings of DEXTER et al. (1996). Other studies found wet gluten content reduced (BOYACIOGLU and HETTIARACHCHY, 1995; MEYER et al., 1986) in artificially *Fusarium* infected samples.

Doug softening was slightly increased in flours exceeding ML in both years; however this effect was not significant (Fig. 6 A). Further

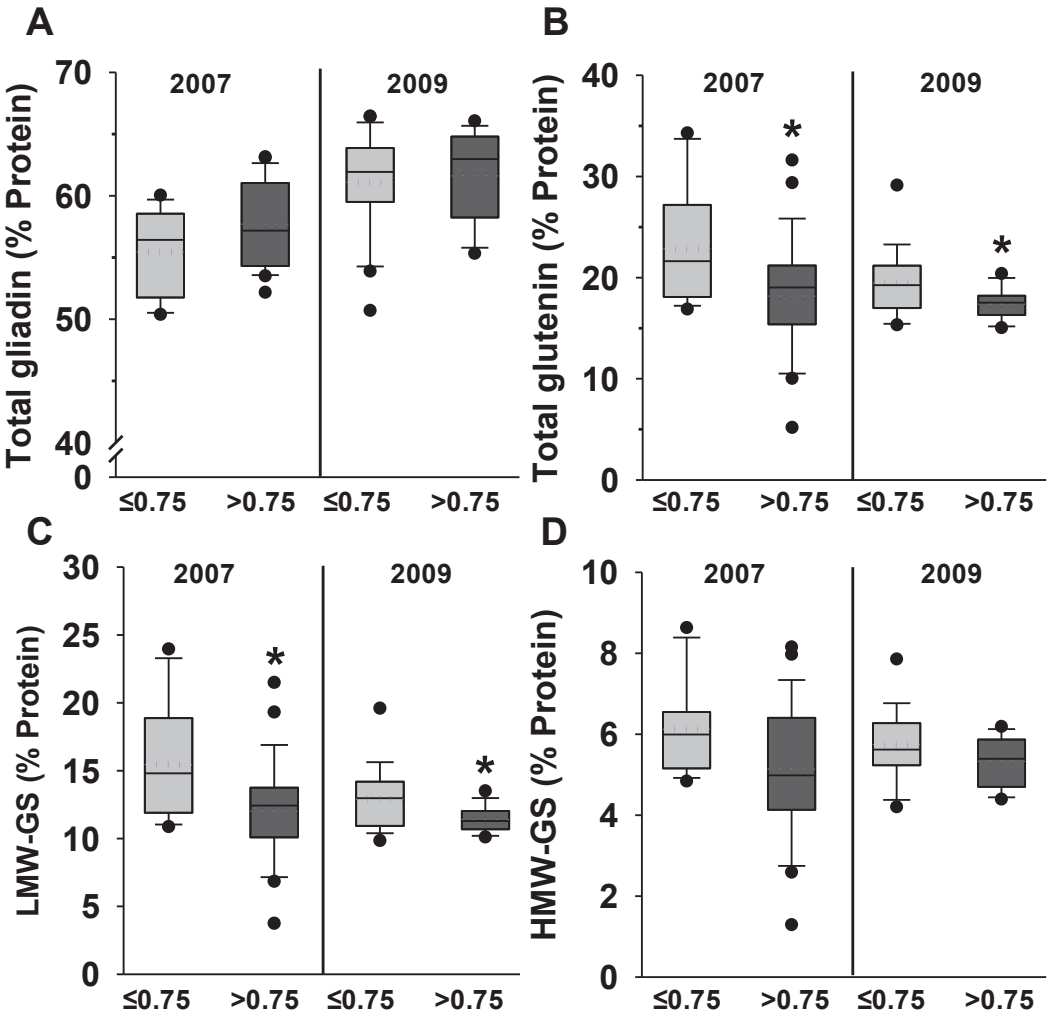

Fig. 4: Total gliadin (A), total glutenin (B), LMW-GS (C), and HMW-GS (D) content of flour Type 550 samples in 2007 and 2009 grouped into fulfilling (≤0.75) and exceeding (>0.75) EU maximum level of DON (mg kg^{-1}) for flour. Significant difference between groups within year is indicated through * ($p<0.001$). Box plots show 25-75% percentile, median (solid line), and mean (dotted line).

dough mixing properties (dough development time, dough stability) did not differ between the two groups (not shown). These results are confirmed by the study of DEXTER et al. (1996). However, other studies reported a decrease of dough development time (WANG et al., 2005b; NIGHTINGALE et al., 1999; PAWELZIK et al., 1998), and dough stability (WANG et al., 2005b; NIGHTINGALE et al., 1999; PAWELZIK et al., 1998) with increasing intensity of *Fusarium* infection. GARTNER et al. (2008) observed cultivar-depended an increase, a decrease as well as no impact of *Fusarium* infection on dough stability; also an increase of dough softening after *Fusarium* infection was observed.

Microbaking test revealed significant differences in baking volume between the years (Fig. 6 B). Baking volume ranged from 360 to 460 ml 100 g^{-1} flour and from 240 to 290 ml 100 g^{-1} flour in 2007 and 2009, respectively. In 2007 baking volume was significantly increased in flours exceeding ML. The difference made up about 44 ml 100 g^{-1} flour between the two groups. In 2009 no difference was detectable. An increase of baking volume after *Fusarium* infection of wheat is a typical observation (LANCOVA et al., 2008; GARTNER et al., 2008; PRANGE et al., 2005; WANG et al., 2005b; DEXTER et al., 1996).

It was observed that loaves prepared from flours highly exceeding ML in 2007 partly lost their shape during leavening. After baking, these loaves appeared wider and flatter (Fig. 7). In these samples dough was already sticky and wet during handling for rapid mix test and therefore could not be brought into the common shape.

Discussion

Agronomic practices influence DON contamination of flour Type 550

Certainly warm and humid weather conditions during wheat anthesis enhanced the epidemic spread of FHB and subsequent DON formation in grain (DE WOLF et al., 2003) and led to extraordinarily high DON content of flour in both years. Over 50% of the total samples of two years exceeded ML. In 2007, 50% of the flours exceeding ML trespassed ML of 0.75 mg kg^{-1} about factor 3.7, in 2009 about factor 2.9 (Fig. 2). The wide range of DON content of flours resulted from to the experimental design of the field trial where grain was harvested. The trial combined agronomic practices such as pre-crop, cultivar choice and fungicide treatment that were all known to influence (reduce or enhance) FHB in wheat. The effect of such agronomic measures on DON content of grain was already investigated thoroughly by e.g. BEYER et al. (2006) and SCHAAFSMA et al. (2001). The present study demonstrates that the effect of pre-crop and cultivar is still observable in processed grain (Fig. 1) even though one could expect that a cleaning step and milling of flour smoothes them (LANCOVA et al., 2008). As in other studies the combination of pre-

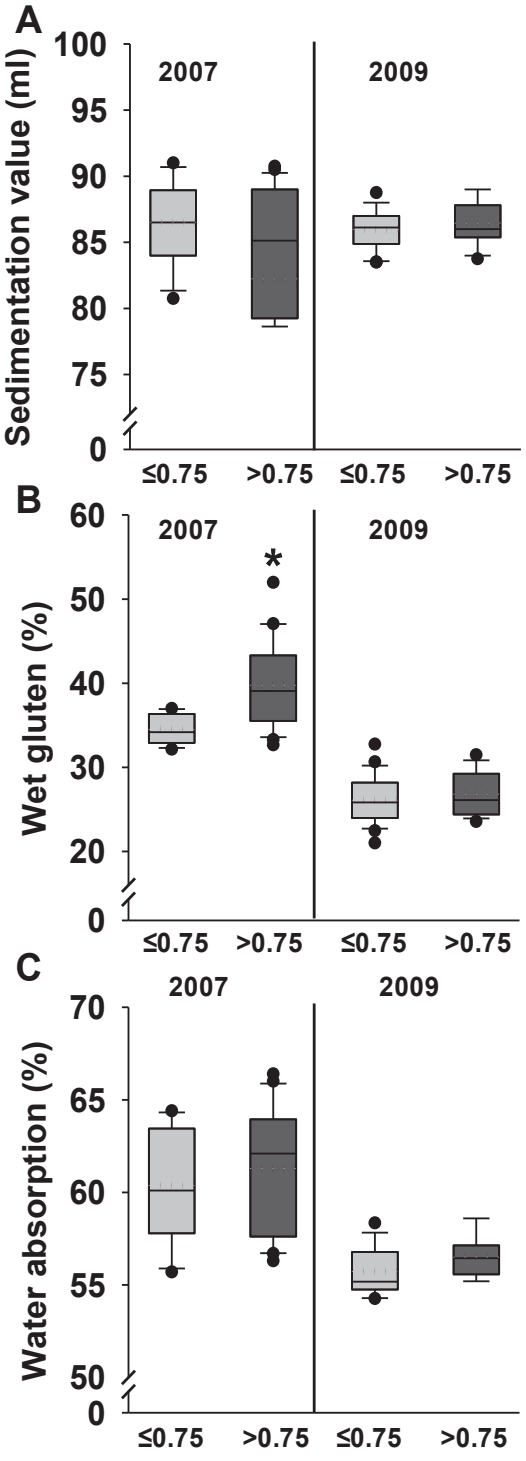

Fig. 5: Sedimentation value (A), wet gluten content (B), and water absorption (C) of flour Type 550 samples in 2007 and 2009 grouped into fulfilling (≤0.75) and exceeding (>0.75) EU maximum level of DON (mg kg^{-1}) in flour. Significant difference between groups within year is indicated through * ($p<0.001$). Box plots show 25-75% percentile, median (solid line), and mean (dotted line).

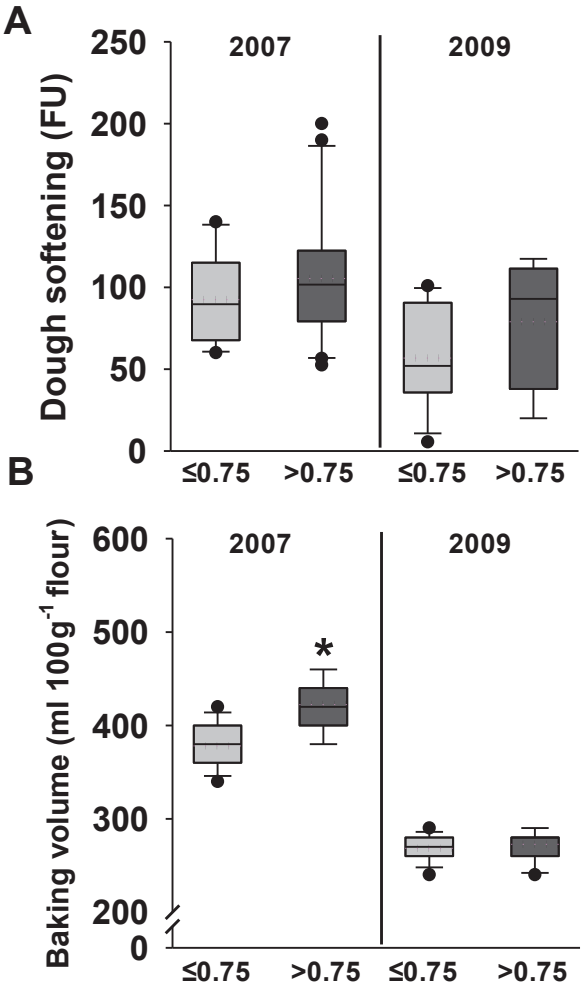

Fig. 6: Dough softening (A) and baking volume (B) of flour Type 550 samples in 2007 and 2009 grouped into fulfilling (≤0.75) and exceeding (>0.75) EU maximum level of DON (mg kg^{-1}) for flour. Significant difference between groups within year is indicated through * ($p<0.001$). Box plots show 25-75% percentile, median (solid line), and mean (dotted line).

crop maize with the highly susceptible cultivar led to the highest DON contamination of flour. Again the high risk coming from pre-crop maize enhancing toxin accumulation in wheat grain is emphasized as reported before (e.g. DILL-MACKY and JONES, 2000; BECK and LEPSCHY, 2000).

High DON contamination of flour is not necessarily associated with reduced technological quality

Aside from mycotoxin contamination of grain and processed cereal, many studies reported on the negative impact of FHB infection of wheat on grain composition, indirect quality traits and baking performance (GARTNER et al., 2008; WANG et al., 2005 a, b; NIGHTINGALE et al., 1999). However studies assessed *Fusarium* damage of wheat and the investigated material differently. This may limit the comparability of the results of this study with former studies and could also explain contradictory results regarding the effect of FHB on wheat composition and quality.

The majority of studies investigated hand-picked kernel fractions with differing degree of FHB damage (SIUDA et al., 2010; NIGHTINGALE et al., 1999; DEXTER et al., 1996; BECHTEL et al., 1985). DON levels of the investigated samples there often highly exceeded those analyzed in this study (NIGHTINGALE et al., 1999). Samples with very high DON contamination might be suitable to reveal what impact FHB has on the wheat kernel, yet they are not so much of practical relevance in bread processing due to legally set percentage of *Fusarium* damaged kernels in grain and ML for DON (DEXTER and NOWICKI, 2003; Commission Regulation (EC) No 824/2000, Commission Regulation (EC) No. 1881/2006). Therefore this study in-

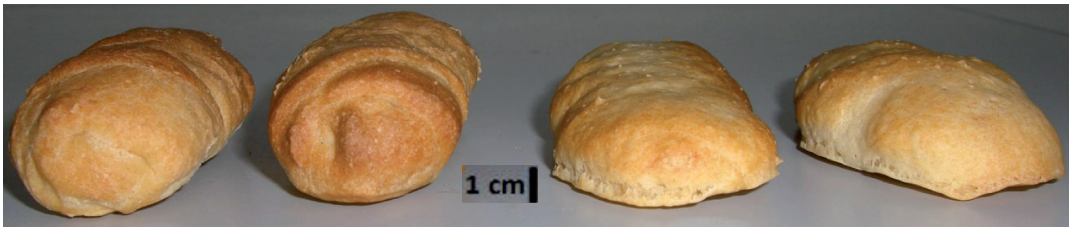

Fig. 7: Bread loaves prepared from flour Type 550 fulfilling (left) and highly exceeding (right) EU maximum level of DON for flour, KREUZBERGER (2008).

vestigated flour from cleaned grain. The still wide range of DON levels in flour made a categorization into flours either fulfilling or exceeding ML possible. Even though grain was cleaned in a one-step procedure, DON contamination of most flours clearly exceeded ML and they were therefore unsuitable for further processing and consumption. Nevertheless it enabled an investigation whether there was an association between composition and quality of flours where DON levels were in and out the legal range.

Baking volume is the complex result of the interaction of various flour components, mainly starch and protein, with water and is the ultimate criterion of wheat to be categorized into wheat quality classes (according to Federal Office of Plant Variety). Starch content, starch properties, protein content and protein composition, more specifically composition and content of gluten protein, are all influenced by the complex interaction of environment and genotype (WILLIAMS et al., 2008; WIESER and KIEFFER, 2001). Even though there were slight differences in starch content and glutenin composition in flours highly exceeding ML (Fig. 3, Fig. 4), baking volume of these flours was not negatively affected. In 2007, baking volume was even increased (Fig. 6). Moreover, indirect quality parameters such as sedimentation volume and protein content (Fig. 5, Fig. 3), which are commonly determined as predictors for baking performance, did not differ between flours fulfilling or exceeding ML indicating that there was no loss of quality to be expected from highly DON contaminated flours. The effect of *Fusarium* infection on baking volume depends on various factors, such as growing season, infection severity, protein content and protein quality (MEYER et al., 1986). DEXTER et al. (1996) explained the increase in baking volume after *Fusarium* infection with an improved viscoelastic balance of gluten. However, overall flours in this study produced very low baking volume not meeting baking wheat quality as one might have expected. Obviously, experimental conditions (e.g. N fertilization) did not meet the demands of the two wheat cultivars to develop their potential as bread wheat.

Very high DON contamination of flour is associated with impaired dough handling and loss of loaf shape

Due to sticky and gluey properties dough handling was difficult in flours highly exceeding ML in 2007, resulting in a loss of loaf shape (Fig. 7). However, this was only visible in flours of the highly susceptible cultivar after pre-crop maize where DON content highly exceeded ML ranging from 3.18 to 11.84 mg kg^{-1}. Loss of shape can also be seen as a loss of quality. However, this is not a commonly measured quality trait. Since dough mixing properties such as dough development time and dough softening did not differ between the two groups (Fig. 6), it became obvious that the dough mixing procedure according to ICC standard must not correspond with dough consistency during further handling. WANG et al. (2005b) also reported an unfavorable dough consistency and handling problems of severely *Fusarium* infected grain. The plastic deformation of dough in this study, which was already visible after the first leavening, was probably due to enzymatic activity during dough processing (NIGHTINGALE et al., 1999; PAWELZIK et al., 1998). Proteolytic activity may result from endoproteases secreted into the kernel by *Fusarium* spp. (PEKKARINEN and JONES, 2002; PEKKARINEN et al., 2000).

Conclusion

Summarizing, except from the loss of shape in 2007, there was no significant loss of technological quality measurable in flours within the range of DON content of this study (up to 11.84 mg kg^{-1}). DON content of flours did not correlate negatively with a significant loss of quality measurable with the common indirect quality parameters or baking test. Loss of loaf shape was only visible in flours highly exceeding ML and therefore anyway unsuitable for further processing and consumption. Since DON content of 0.75 mg kg^{-1} is the criterion of exclusion for further processing of flour, it can be concluded that technological quality of flours fulfilling ML is not significantly impaired by FHB of grain and that DON content must exceed ML manyfold before changed flour composition and reduced quality due to FHB infection of grain becomes measurable in flour Type 550 with the common methods.

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