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Distribution of Trichothecenes, Zearalenone, and Ergosterol in a Fractionated Wheat Harvest Lot

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To investigate possible co-occurrences of type B trichothecenes and zearalenone within a *Fusarium culmorum*-infected wheat harvest lot, kernels were fractionated into six groups by visual criteria. The *Fusarium*-damaged kernels were subdivided into white, shrunken, and red kernel groups, and the remaining kernels were sorted into healthy, black spotted, and nonspecific groups. The distribution patterns of nivalenol, deoxynivalenol, zearalenone, and ergosterol were determined for possible correlations. Significant correlations between the distribution patterns were found for the mycotoxins and ergosterol for the grouped kernels ($r = 0.997-0.999$, $p < 0.0001$). Additionally, remarkably outstanding levels of nivalenol (24-fold more than the mean at 1.16 mg/kg), deoxynivalenol (27-fold more than the mean at 0.16 mg/kg), zearalenone (25-fold more than the mean at 77 μg/kg), and ergosterol (17-fold more than the mean at 13.4 mg/kg) were found in the red kernel group. Further, detailed mycotoxin and ergosterol analyses were carried out on various segments (kernel surface, conidia, bran, and flour) of the red kernels. However, the mycotoxin and ergosterol distribution profiles revealed nonsignificant correlations for these kernel segments, with the exception of deoxynivalenol and nivalenol, which were moderately correlated ($r = 0.948$, $p = 0.035$).

KEYWORDS: Mycotoxins; *Fusarium*-damaged kernels; ergosterol; wheat; correlation; HPLC-FLD/DAD

INTRODUCTION

Fusarium head blight (FHB) or scab, caused by diverse species of the genus *Fusarium*, is an important disease of grain crops worldwide. FHB not only causes harvest losses, but also is associated with the production of mycotoxins known to cause a broad spectrum of acute and chronic diseases in humans and livestock (*1*). The starting point for this causal chain is the use of contaminated grain as the basic material for animal feeds and in the production of human food products (*2*).

Fusarium-infected grains often are contaminated with mycotoxins of the trichothecene, fumonisin, and zearalenone family. All these mycotoxins are products of secondary metabolism and mostly originate from different biosynthetic pathways. For instance, the two most common, trichothecenes deoxynivalenol and nivalenol, are derived from farnesyl pyrophosphate; on the other hand, zearalenone is produced via the acetate−polymalonate pathway (*3, 4*). It is also known that the trichothecenes and zearalenones have different biological functions in the life cycles of the fungus. Deoxynivalenol, for instance, which is a potent protein synthesis inhibitor, is postulated to act as an inhibitor of plant defense response genes (*5, 6*). However, zearalenone induces sporulation and regulates the sexual stages of the fungus (*7, 8*). Several studies have reported that the biosynthesis of the trichothecenes and zearalenone does not occur simultaneously (*9, 10*). Deoxynivalenol, for instance, is produced in high concentrations during the earlier stages of the life cycle, whereas zearalenone is formed at a later stage.

In spite of having apparently different biosynthetic origins and specific functions, zearalenone and the trichothecenes deoxynivalenol and nivalenol are sometimes found to coexist in contaminated grains (*11–15*). There have been several conflicting reports regarding a possible correlation between the occurrence of trichothecenes and zearalenone (*16–18*). Most of these studies were based on correlation profiles and comparative data sets derived from various samples of mold-infected corn (*19*).

However, the aim of our study was to investigate whether the occurrence of both types of mycotoxin within a single wheat harvest lot was a correlated event. Therefore, the kernels contaminated with zearalenone, nivalenol, and deoxynivalenol were fractionated into six different groups. The kernels were grouped according to their visual attributes. The *Fusarium*-damaged kernels (FDKs) were subdivided into white, shrunken, and red kernels, and the non-FDKs were sorted into healthy, black spotted (symptoms of a possible infection with *Alternaria* or *Cladosporium* species (*20*), and remaining kernel groups. For each group the mycotoxin content was determined. The resulting distribution patterns of the mycotoxins were then compared to identify any possible correlations in their occurrence.

Further analyses were carried out with the red kernel group to improve the distribution profile of zearalenone, nivalenol,

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and deoxynivalenol. At this point, extracts of the bran, flour, kernel surface, and conidial suspension were investigated. Additionally, the ergosterol content of all fractions was determined to correlate the mycotoxin content to the corresponding levels of fungal biomass (*21–24*).

The possibility of a correlated occurrence of trichothecenes and zearalenone within a contaminated grain harvest can be used to improve existing decontamination methods or to develop new strategies for the efficient removal of both types of mycotoxin simultaneously. The occurrence of conidia, representing an additional risk to human health, was also monitored for all groups.

MATERIALS AND METHODS

Chemicals. A 20 kg lot of a naturally *Fusarium culmorum*-infected wheat harvest was investigated in our study. Zearalenone, nivalenol, and deoxynivalenol were obtained from Biopure (Tulln, Austria). Ergosterol was obtained from Sigma-Aldrich (Hamburg, Germany). Acetonitrile (HPLC-grade) and hexane (p.a) were purchased from J.T. Baker (Griesheim, Germany), and bidistilled water was produced utilizing a Milli-Q synthesis A 10 system (Millipore, Billerica, MA).

Fungus Identification. A 1 g portion of ground wheat kernels was suspended in 10 mL of sterile saline and plated onto Difco malt extract agar. After incubation at 26 °C for 7 days individual colonies were isolated from the plate and prepared for DNA extraction as described by Tintelnot et al. (*25*). The fungus was identified by PCR using a Biometra Cycler Uno II (Biometra GmbH, Göttingen, Germany) with the primers ITS4 and ITS5 according to White et al. (*26*). The sequence analysis of the internal transcribed spacer (ITS) regions inside the nuclear rDNA revealed *F. culmorum*, identical to the sequences of several isolates of *F. culmorum* deposited by Mishra et al. (*27*).

Sample Preparation for Mycotoxin Analysis. A 3 kg random sample lot of the 20 kg wheat kernel harvest was manually fractionated into six visually distinguishable groups: (1) red kernels, (2) white kernels, (3) kernels with black spots, (4) shrunken kernels, (5) healthy kernels, (6) remaining kernels. The red, white, and black spotted kernels were regular in shape. The remaining group contained healthy characteristics but with slightly mixed properties of groups 2, 3, and 4. To calculate the percentage by mass of each group related to the total mass of kernels, a random sample of 50 g of the entire batch was sorted into the defined groups. The mean was obtained after five replications.

An aliquot of about 20 g of each kernel group was ground in a ZM 1000 centrifugal mill (Retsch Technology GmbH, Haan, Germany) into 1 mm fractions. The ground samples of 5 g from each group were extracted with 25 mL of acetonitrile/water (84:16, v/v) on a KS 500 laboratory shaker (Janke & Kunkel IKA-Werke GmbH, Stauffen, Germany) for 1 h at 180 rpm. The supernatant was filtered through filter paper (Macherey-Nagel, Düren, Germany). For the cleanup procedure, an aliquot (8 mL) was applied to a MultiSep column (225 Trich for deoxynivalenol and nivalenol and 226 AflaZon for zearalenone, Romer Laboratories Diagnostik GmbH, Tulln, Austria) and eluted at a flow rate of approximately 1 mL/min. Before MultiSep 226 AflaZon column cleanup for zearalenone analysis the 8 mL aliquot was spiked with 8 μL of acetic acid. The eluate was evaporated to dryness using a rotary evaporator for 30 min at 40 °C. The residue was dissolved in 500 μL of acetonitrile/water (60:40, v/v) for analysis of zearalenone and in 500 μL of acetonitrile/water (10:90, v/v) for nivalenol and deoxynivalenol analysis. Aliquots of 100 μL were injected into the HPLC-FLD/DAD system. All HPLC analyses were carried out in triplicate.

Sample Preparation for Ergosterol Analysis. The isolation of ergosterol was carried out according to the method of Dong et al. (*23*). In a 250 mL round-bottom flask equipped with a reflux condenser, a 1 g ground sample of each kernel group was extracted with 20 mL of 10% KOH/MeOH (w/v) solution for 60 min at 80 °C. After the addition of 5 mL of water the sample was filtered and the ergosterol extracted with 25 mL of hexane. The phases were separated using a separating funnel. A 5 mL aliquot of the hexane extract was dried under nitrogen. For HPLC analysis the residue was dissolved in 500 μL of methanol.

Preparation of Red Kernel Samples for Analysis. The sample preparation for the mycotoxin analysis of the red kernels was carried out in three steps. First, 1 g of wheat kernels was extracted with 20 mL of acetonitrile/water (84:16, v/v) on a laboratory shaker for 1 h at 180 rpm. Second, the extracted kernels were dried in air and fractionated in bran and flour by gently milling with a pestle and mortar. The flour was separated from the bran using a 2 mm test sieve (Retsch, Germany). Third, the flour and bran were extracted separately in 20 mL of acetonitrile/water (84:16, v/v) on a laboratory shaker for 1 h at 180 rpm. For the cleanup procedure, an 8 mL aliquot of the filtered extracts was applied to the MultiSep column 225 Trich for deoxynivalenol and nivalenol and the 226 AflaZon column for zearalenone. After evaporation of the solvent the residues were dissolved in 500 μL of acetonitrile/water (60:40, v/v) for the analysis of zearalenone and 500 μL of acetonitrile/water (10:90, v/v) for nivalenol and deoxynivalenol analyses. A 100 μL sample was injected into the HPLC-FLD/DAD system.

The preparation of the bran and flour for ergosterol analysis of the red kernels was identical to that for the mycotoxin analysis. The extraction of wheat kernels, bran, and flour was carried out according to the procedure used for the ground samples. All analyses were carried out in triplicate.

To obtain a conidia sample, 1 g of wheat kernels was extracted with 4 mL of bidistilled water for 3 min in an ultrasonic bath. The water suspension was removed with a Pasteur pipet, transferred to a 10 mL screw top vial, and dried under nitrogen. Samples used for the detection of mycotoxin and ergosterol were prepared and analyzed as described above. The analyses were carried out in triplicate.

Zearalenone Analysis. Samples were analyzed with an HPLC-FLD/DAD 1200 series system from Agilent Technologies (Waldbronn, Germany) equipped with a 250 mm × 4 mm i.d., 5 μm, Eurospher-100 RP-18 column (Knauer, Berlin, Germany). The chromatographic separation was achieved using the following parameters: solvent A, acetonitrile; solvent B, water; linear gradient, 60−100% acetonitrile in 14 min; flow rate, 1.0 mL/min; injection volume, 100 μL. Fluorescence detection of zearalenone was carried out at an excitation wavelength of 271 nm and an emission wavelength of 452 nm.

Nivalenol and Deoxynivalenol Analysis. Samples were analyzed with an HPLC-FLD/DAD 1200 series system from Agilent Technologies equipped with the same column as for zearalenone analysis. The following chromatographic program was used: solvent A, water; solvent B, acetonitrile; linear gradient, 10−100% acetonitrile in 23 min, 2 min hold, 100−10% acetonitrile in 7 min; flow rate, 0.7 mL/min; injection volume, 100 μL. Nivalenol and deoxynivalenol were detected using a diode array detector at 224 nm.

Ergosterol Analysis. Samples were analyzed with an HPLC-FLD/DAD 1200 series system from Agilent Technologies equipped with the same column as for zearalenone analysis. The chromatographic separation was achieved using the following parameters: solvent A, methanol/0.05% acetic acid (90:10, v/v); solvent B, methanol; gradient procedure, 10% solvent B for 10 min, from 10% to 100% solvent B in 10 min, 5 min hold of 100% solvent B, from 100% to 10% solvent B in 15 min; flow rate, 1.0 mL/min; injection volume, 100 μL. Ergosterol was detected using a diode array detector at 282 nm.

Method Validation. The HPLC sample signals were identified by comparing the retention times against those of known standards of zearalenone, nivalenol, deoxynivalenol, and ergosterol. The analyses were verified by their typical UV spectra generated in the single-peak spectral mode of the diode array detector. The peak purity of the analyzed mycotoxin and ergosterol signals was controlled by recording the DAD single-peak spectra among the integrated peak width (sampling rate one spectrum/0.03 min, resulting in about 10 spectra per peak).

The content of mycotoxin and ergosterol was calculated from the calibration curves generated by calculation of the peak areas of standard solutions (four concentration levels, three injections per level). The precision of the mycotoxin and ergosterol methods was reflected by the values of the standard deviations, which were established by performing the same procedure five times on the unsorted kernel

Table 1. Mycotoxin Content of the Grouped Kernels[a]

grouped kernels	percentage by mass of the total number of kernels,[b] % ± *s*	zearalenone content, μg/kg ± *s*	nivalenol content, mg/kg ± *s*	deoxynivalenol content, mg/kg ± *s*
red	1.1 ± 0.2	1952 ± 214	27.73 ± 4.16	4.39 ± 0.79
shrunken	3.1± 0.2	149 ± 16	1.31 ± 0.20	0.40 ± 0.07
white	2.3 ± 0.3	119 ± 13	3.33 ± 0.50	0.34 ± 0.06
black spotted	1.5 ± 0.2	67 ± 7	0.52 ± 0.08	0.25 ± 0.05
healthy	3.5 ± 0.3	9[c] ± 1	0.29 ± 0.04	0.14[c] ± 0.03
remaining	88.5 ± 0.5	46 ± 5	0.65 ± 0.10	0.06[c] ± 0.01
unsorted[d]	100	77 ± 8	1.16 ± 0.17	0.16 ± 0.03

[a] Data were corrected for recovery: zearalenone (88%), nivalenol (81%), deoxynivalenol (80%). *s* = standard deviation. [b] Mean of five calculations. [c] <LOQ (zearalenone, 17 μg/kg; deoxynivalenol, 0.157 mg/kg). [d] Random sample, taken directly from the wheat harvest lot.

samples. The recovery was received from the recovery function. Four 5 g milled samples of unsorted kernels were used to prepare one unspiked probe and three spiked probes for each of the compounds under study. Samples were spiked with 46, 94, and 142 ng/g zearalenone, 498, 791, and 1023 ng/g nivalenol, 92, 213, and 307 ng/g deoxynivalenol, and 182, 365, and 546 ng/g ergosterol. The mycotoxin and ergosterol concentrations (mean of three injections) of the unspiked and spiked samples were plotted as a function of the spiked concentration. The slope of the recovery function revealed that 88% zearalenone, 81% nivalenol, 80% deoxynivalenol, and 83% ergosterol were recovered. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated according to the calibration method described in the DIN (German Institute for Standardization) 32645 guidelines (*37*) and the procedure proposed by the International Union of Pure and Applied Chemistry (IUPAC) (*39*). For zearalenone samples, the LOD was 4 μg/kg and the limit of quantification (LOQ) was 17 μg/kg. The LOD of nivalenol was 37 μg/kg, and the LOQ was 142 μg/kg. The LOD and LOQ for deoxynivalenol were 41 and 157 μg/kg, respectively, and for ergosterol the LOD and LOQ were 0.9 and 3.8 mg/kg, respectively.

Morphological Features of the Fungus. *Fusarium* conidia and hyphae were counted in a Neubauer chamber using a phase contrast dark field microscope (Zeiss Axioskop, Jena, Germany). Samples of 1 g of each kernel group were extracted in 4 mL of bidistilled water for 3 min in an ultrasonic bath. The suspension was removed with a Pasteur pipet, transferred to a 10 mL screw top vial, and dried under nitrogen. The dried extract was redissolved in 1 mL of 0.85% (w/v) NaCl solution, and the chamber was filled with 2 × 7.5 μL of NaCl solution. The analysis was carried out in triplicate. On the basis of intralaboratory studies, the relative standard deviation of the number of fungal morphological elements was approximately 10%.

RESULTS AND DISCUSSION

In our study kernels of a low-quality wheat harvest lot, containing 6.5% FDKs, were sorted into six visually distinguishable groups. The mycotoxin analysis revealed a nonhomogeneous distribution among these classified groups (**Table 1**). An outstandingly high level of mycotoxin was found in the group of kernels having a red appearance. The mycotoxin content of these groups was 24−28 times greater than the corresponding mean content, represented by the unsorted kernel sample (**Figure 1**). However, the mycotoxin content of the black, shrunken, white, and remaining groups fell into a range similar to the mean levels. The contents of zearalenone and nivalenol were, respectively, 9 and 4 times lower in the group of healthy kernels than their mean levels. The range of deoxynivalenol concentrations in this group centered around the mean value.

The content of ergosterol as a marker of fungal biomass was determined for all groups. The distribution of ergosterol was very similar to that of the mycotoxins. The red kernel group exhibited a remarkably high level of ergosterol, which at 228 mg/kg was 17 times higher than the mean concentration (**Figure 2**). The ergosterol content in the healthy kernels was only half the mean level.

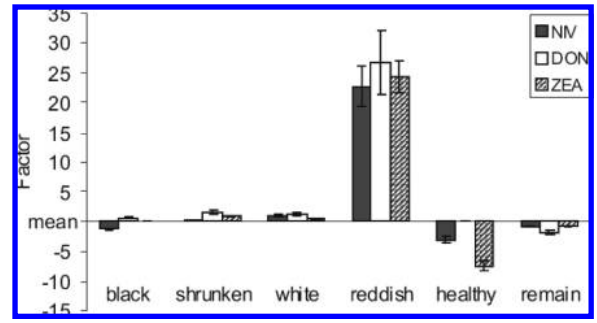

Figure 1. Increases or decreases (illustrated by factors) in the mycotoxin content of the kernel groups related to the mean value. The mean content of mycotoxins was determined by analyses of the unsorted kernel probe. The unsorted kernel probe was taken at random from the wheat harvest lot.

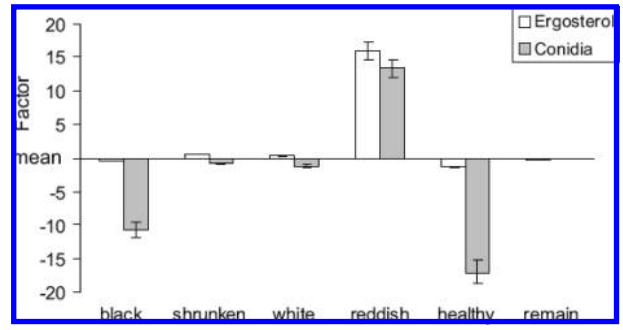

Figure 2. Increases or decreases (illustrated by factors) in the level of ergosterol and number of conidia in the kernel groups related to the mean value. An unsorted kernel probe was used to establish the mean ergosterol and conidia content.

To examine mycological occurrence on the kernel surface, the number of conidia and hyphae fragments were determined for all groups of kernels. Using a Neubauer chamber, conidia and hyphae fragments were identified and counted using a phase contrast dark field microscope. In addition to a high concentration of mycotoxins, the red kernel group also contained a particularly high level of conidia (**Table 2**). The number of conidia (2.2×10^7 conidia/g) was 14 times greater than the mean level in the unsorted sample (**Figure 2**). The high number of conidia was proportional to the ergosterol content in the red kernel group, but was significantly reduced, which is the reverse situation for ergosterol, in the black spotted and healthy groups (**Figure 2**). The kernels in the black spotted and healthy groups possessed, respectively, 12 and 18 times fewer conidia than the mean values. The number of conidia found in the shrunken, white, and remaining groups was similar in magnitude to the

Table 2. Morphological Elements and Ergosterol Content of the Grouped Kernels[a]

grouped kernels	morphological elements of *F. culmorum*		
	number of conidia, 10^4/g	number of hyphae, 10^4/g	ergosterol content, mg/kg
red ($n = 176$)	2200 ± 220	nd	228 ± 18
shrunken ($n = 180$)	82 ± 8.2	4.4 ± 0.5	22.2 ± 1.8
white ($n = 190$)	69 ± 6.9	2.1 ± 0.2	18.4 ± 1.5
black spotted ($n = 264$)	13 ± 1.3	4.3 ± 0.4	9.4 ± 0.8
healthy ($n = 236$)	8.5 ± 0.9	5.1 ± 0.5	6.0 ± 0.5
remaining ($n = 200$)	140 ± 1.4	4.0 ± 0.4	10.6 ± 0.9
unsorted[b] ($n = 210$)	153 ± 1.5	3.9 ± 0.4	13.4 ± 1.1

[a] Data for ergosterol were corrected for recovery (83%). n = counted squares in the Neubauer chamber, and nd = not detected. [b] Random sample, taken directly from the wheat harvest lot.

Table 3. Mycotoxin and Ergosterol Content within the Fractions of the Red Kernels

fraction of red kernels	zearalenone content, μg/kg	nivalenol content, mg/kg	deoxynivalenol content, mg/kg	ergosterol content, mg/kg
conidia[a]	21	2.3	0.22	3.2[b]
surface[a]	592	2.5	0.46	104
bran	1181	24.6	4.30	113
flour	1282	24.9	2.87	136

[a] Mycotoxin and ergosterol contents of the conidia and the surface fractions were related to the whole kernel mass. [b] <LOQ (ergosterol, 3.8 mg/kg).

mean value. *Fusarium* hyphae were found in similar amounts in all groups. Apart from the red kernel group, the group contained only conidia as the single morphological element (**Table 2**).

In the second approach the red kernel group was examined in detail. Extracts of the kernel surface, conidia, bran, and flour were analyzed for mycotoxin and ergosterol content. After surface extraction the kernels were dried and separated, resulting in a mean percentage mass of 55% bran and 45% flour. The concentrations of mycotoxin and ergosterol were calculated taking into account the percentage masses of the fractions (**Table 3**). The conidia were obtained by water extraction of the whole kernels. Microscopic examination of the extract showed the exclusive occurrence of conidia as the only fungal element in the red kernel group. To eliminate the possibility that some of the mycotoxins were of nonconidial origin, the extract was filtered and the conidia-free aqueous filtrate was analyzed for mycotoxin content. All the aqueous samples examined were below the LOD for the mycotoxins under investigation.

For zearalenone and ergosterol the flour fractions showed the highest content compared to the surface and bran fractions. However, the highest deoxynivalenol content was found in the bran, in accord with the findings of Young et al. (*38*). The nivalenol content was approximately equal in the bran and flour. A significant reduction in mycotoxins was found in the conidia fraction. The zearalenone, deoxynivalenol, and nivalenol contents of the conidia were 92, 20, and 12 times lower than their mean values, respectively. The ergosterol content of the conidia in the red kernel group was 60 times lower than the mean value.

Figure 3 shows the percentage distribution of the total content of mycotoxins and ergosterol within the red kernel fractions. Nivalenol made up 50% and deoxynivalenol 58% of the total mycotoxin content in the kernel bran. The surface extracts contained only 9% nivalenol and 12% deoxynivalenol. However, the kernel surface contained 32% zearalenone and the bran 36%. A total of 92% of the 9% nivalenol content of the surface fraction originated from the conidia. A total of 48% of the 12%

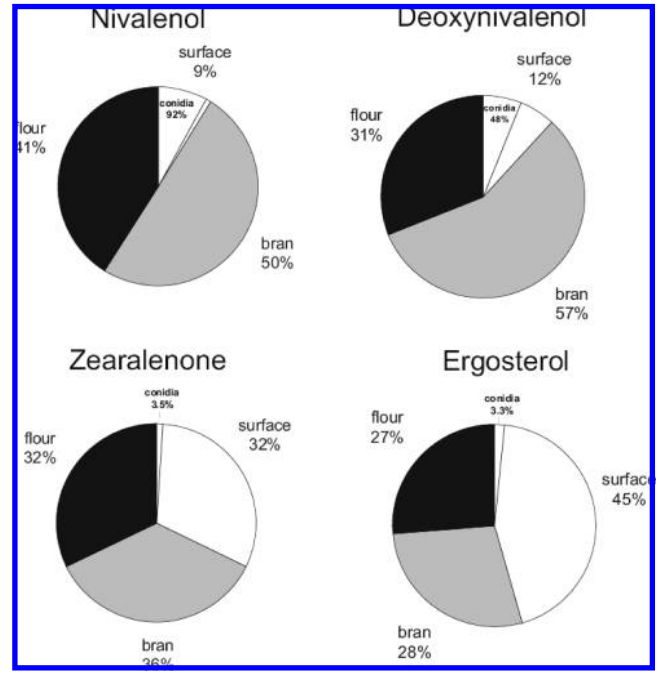

Figure 3. Percentage of total mycotoxins and ergosterol present in the fractions of the red kernels. The values shown for the conidia are a percentage of the total surface values.

deoxynivalenol content extracted from the kernel surface was located in the conidia. However, only 3.5% and 3.3% of the surface content of zearalenone and ergosterol was contributed by the conidia. The flour fractions were contaminated with 31% deoxynivalenol, 41% nivalenol, and 32% zearalenone of the total mycotoxin content. Of the total ergosterol content 45% was found in the surface extract and 28% and 27% was found in the bran and flour, respectively.

To determine a possible correlation between the mycotoxins and ergosterol for the fractionated kernel groups, a null hypothesis significance test was applied (*28*). The following parameters were chosen: expected correlation, 0; H_0, null hypothesis, the correlation was about the same as the expected value; H_a, alternative hypothesis, the correlation was quite different from the expected value; sample size, $n = 7$; level of significance, $\alpha = 0.05$; hence, the null hypothesis was rejected ($p \leq \alpha$).

For zearalenone/nivalenol, the correlation coefficient $r = 0.997$, for zearalenone/deoxynivalenol, $r = 0.999$, for nivalenol/deoxynivalenol, $r = 0.997$, for zearalenone/ergosterol $r = 0.999$, for nivalenol/ergosterol, $r = 0.997$, and, for deoxynivalenol/ergosterol, $r = 0.999$; for all combinations, $p < 0.0001$. There was very strong evidence against the null hypothesis, which was therefore rejected. The alternative hypothesis was accepted. The data provided statistical evidence to show that the presence of the various mycotoxins was correlated and that their occurrence was also related to the presence of ergosterol. These results confirmed several studies concerning significant correlations between fungal biomasses, as estimated by ergosterol and mycotoxin concentrations (*23, 29*).

For all distribution profiles the red kernel group contained the highest concentration of all mycotoxins and ergosterol. Within the contaminated wheat harvest lot, the red kernels represent the most severe contamination, and a commercial high-speed optical sorter is already in use for the removal of these red kernels (*30*). Additionally, the high concentration of conidia associated with this group will also be reduced with this technique. Consequently, a further potential health risk due to

chronic respiratory intoxication can be reduced by removing the airborne conidia associated with the rejected red kernels (*31, 32*). Removal of the conidia will also reduce the impact of conidial mycotoxins, which most likely play an important role in enhancing these disease symptoms (*33*). In this regard, we observed a substantial mycotoxin concentration in the conidia examined in our study: conidia (about 2×10^7) extracted from 1 g of red kernels contained 21 ng of zearalenone, 220 ng of deoxynivalenol, and 2300 ng of nivalenol.

Comparing the mycotoxin distribution profiles for all kernel groups, detailed analysis revealed differences in the distribution patterns of trichothecenes and zearalenone in the various fractions of the red kernels. The established null hypothesis was also used to test for a possible correlation between the mycotoxins and ergosterol within the fractions of red kernels. With the expected correlation equal to 0 (H_0, null hypothesis, the correlation was about the same as the expected value; H_a, alternative hypothesis, the correlation was quite different from the expected value) and the sample size $n = 4$, the level of significance $\alpha = 0.05$; therefore, the null hypothesis was rejected ($p \leq \alpha$).

There was moderate evidence against the null hypothesis for nivalenol/deoxynivalenol ($r = 0.948$, $p = 0.035$), which was rejected; however, a weak correlation was accepted. There was little or no real evidence against the null hypothesis for zearalenone/nivalenol ($r = 0.643$, $p = 0.222$), zearalenone/deoxynivalenol ($r = 0.676$, $p = 0.205$), nivalenol/ergosterol ($r = 0.157$, $p = 0.437$), or deoxynivalenol/ergosterol ($r = 0.237$, $p = 0.405$) and only tentative evidence against the null hypothesis for zearalenone/ergosterol ($r = 0.857$, $p = 0.099$). The null hypothesis was not rejected for all the pairs investigated, except nivalenol/deoxynivalenol. Therefore, with the exception of nivalenol/deoxynivalenol, no correlation was found between the presence of mycotoxins and ergosterol in the various fractions of the red kernels.

Similar distribution profiles were found for nivalenol and deoxynivalenol on the kernel surface and in the bran and flour. The majority of both mycotoxins was found in the bran (50% and 57%); however, only 9% nivalenol and 12% deoxynivalenol were associated with the kernel surface. On the other hand, of the total mycotoxins found on the kernel surface zearalenone accounted for 32%. Similar to zearalenone, the majority of the ergosterol was found on the kernel surface (45%). The conidia contained only 3.3% and 3.5%, respectively, of the total ergosterol and zearalenone content of the surface fraction. From this it follows that further fungal biomass of nonconidial origin must be present on the surface of the red kernel and accounts for about 1/3 of the total zearalenone content of that kernel group.

In conclusion, two different wheat kernel fractionations were carried out in our study. First, the wheat harvest lot was sorted into six visually distinguishable groups. Second, the group containing the red kernels was fractionated into different kernel components. All fractions were analyzed for their mycotoxin content, and distribution profiles for the mycotoxins were generated. Similar distribution patterns were found for the trichothecenes and zearalenone in the fractionated kernel groups. However, the distribution patterns of the red fractionated kernel components showed variances. Despite this, the results of both fractionation experiments can be used to develop methods to improve the simultaneous decontamination of trichothecenes and zearalenone from *Fusarium*-infected kernels.

In our case, for instance, rejection of the red kernels resulted in a significant reduction in both types of mycotoxins. However, if the decontamination procedure is carried out by pearling, washing, or peeling (*34, 35*) the red kernels, the concentrations of zearalenone and trichothecenes would probably not be equally reduced. However, by removing the outer part of the kernel in a dehuller, for instance (*36*), both types of mycotoxins can be reduced by a similar percentage. Thus, further intraharvest analyses of grain samples contaminated with zearalenones and trichothecenes should be carried out to optimize the various approaches to decontamination so that the concentrations of both types of mycotoxins are reduced simultaneously.

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