



Determination of Deoxynivalenol and Zearalenone in Single Kernels From a Highly Contaminated Corn Sample

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Abstract

Corn, when exposed to wet growing conditions in the presence of *Fusarium* mold species, can be contaminated with both deoxynivalenol and zearalenone mycotoxins. These mycotoxins commonly contaminate corn separately, but can also occur together. A corn sample highly contaminated with both deoxynivalenol (15.9 ppm) and zearalenone (1.5 ppm) was found in Southern Ontario, Canada during the 2006 growing season. Individual kernels of this corn were analyzed separately to determine the distribution of both deoxynivalenol and zearalenone in the same kernel. A single kernel of corn was crushed, extracted with acetonitrile/water, and purified with a solid phase cleanup column. The purified extract was divided into 2 portions. One portion was analyzed for zearalenone by HPLC using fluorescence detection and the other portion, after additional purification, was analyzed for deoxynivalenol by HPLC using UV detection. Approximately 100 kernels were selected for analysis and separated into 3 groups based on their physical appearance; normal kernels, slightly damaged kernels, and highly damaged kernels. On average, the individual kernel weights decreased while the deoxynivalenol and zearalenone increased concentration with the amount of physical damage observed. For the normal kernel group, 34% were positive for deoxynivalenol (range from 0.1 ppm to 7.0 ppm) and 6% positive for zearalenone (range from 0.1 ppm to 0.9 ppm). For the slightly damaged kernels, the number of positives for deoxynivalenol and zearalenone was 91% (range from 0.4 ppm to 424.1 ppm) and 76% (range from 0.1 ppm to 102.1 ppm) respectively. For the highly damaged kernels, the number of positives for deoxynivalenol and zearalenone was 94% (range from 0.2 ppm to 699.0 ppm) and 96% (range from 0.1 ppm to 221.5 ppm) respectively. The number of kernels contaminated with both deoxynivalenol and zearalenone greater than 0.1 ppm each for the normal, slightly damaged, and highly damaged kernel groups were 0%, 69%, and 89% respectively. There was no correlation in the concentration of deoxynivalenol and zearalenone in the single kernels for either group.

Procedure

Individual kernels from a highly contaminated DON (15.9 ppm) and zearalenone (1.5 ppm) corn sample were divided into 3 groups of approximately 35 kernels each depending on their physical appearance; normal, slightly damaged, and highly damaged kernels.

Each kernel was analyzed separately for DON and zearalenone by HPLC.

The individual kernels were crushed and extracted with acetonitrile/water (84/16) by vortexing for 3 minutes and purified by a solid phase cleanup column (Trilogy TC-M160).

The purified sample extract was divided into 2 portions.

Zearalenone Analysis

A portion of the purified extract was evaporated, reconstituted in mobile phase, and analyzed by HPLC with fluorescence detection.

- HPLC Conditions
 - Column : Reversed Phase C-18
 - Mobile Phase: water/acetonitrile/methanol (25/10/10)
 - Flow Rate: 2.0 ml/min
 - Fluorescence Detector: Excitation 285 nm, Emission 460 nm

DON Analysis

A portion of the purified extract was further purified using a solid phase cleanup column (Trilogy TC-T200), evaporated, reconstituted in mobile phase, and analyzed by HPLC with UV detection

- HPLC Conditions
 - Column: Reversed Phase C-18
 - Mobile Phase: water/methanol/acetonitrile (92/4/4)
 - Flow Rate: 1.0 ml/min
 - UV Detector: 220 nm



Group A - Normal Corn Kernels			
Kernel Weight(g)	DON ppm	Zearalenone ppm	Mean
1	0.43	0.00	15.34
2	0.39	0.00	2.34
3	0.50	0.00	0.37
4	0.39	0.00	0.39
5	0.34	0.00	0.34
6	0.34	0.00	0.34
7	0.45	1.27	0.45
8	0.38	0.65	0.38
9	0.37	0.81	0.37
10	0.41	0.00	0.41
11	0.43	0.00	0.43
12	0.48	0.00	0.48
13	0.37	0.00	0.37
14	0.45	1.34	0.45
15	0.47	1.23	0.47
16	0.45	0.00	0.45
17	0.33	0.00	0.33
18	0.46	0.00	0.46
19	0.43	0.00	0.43
20	0.38	0.82	0.38
21	0.43	0.00	0.43
22	0.42	2.58	0.42
23	0.44	0.00	0.44
24	0.40	0.00	0.40
25	0.39	0.00	0.39
26	0.48	7.02	0.48
27	0.40	0.00	0.40
28	0.35	0.00	0.35
29	0.54	0.00	0.54
30	0.39	0.00	0.39
31	0.41	0.00	0.41
32	0.42	0.00	0.42
33	0.39	0.12	0.39
34	0.39	2.63	0.39
35	0.34	0.00	0.34
Mean	0.41	0.69	0.63

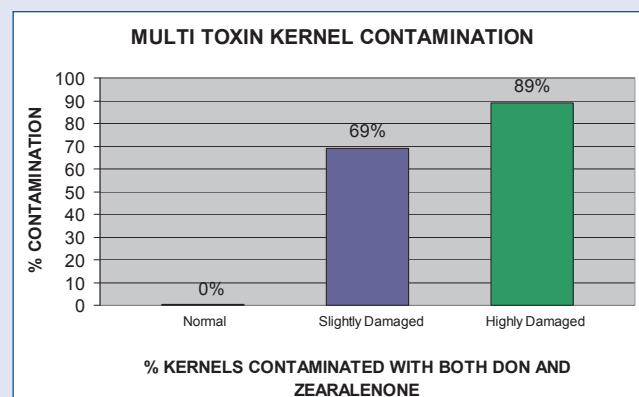
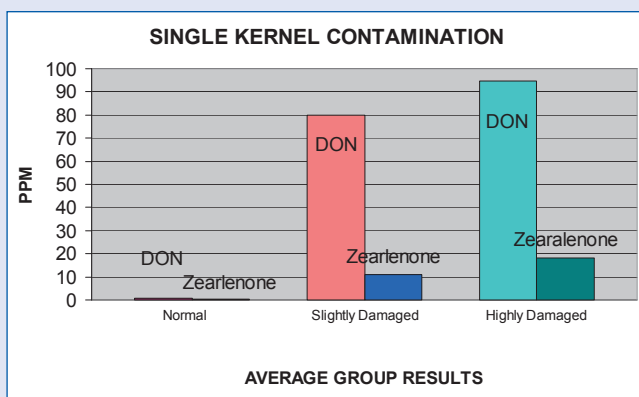
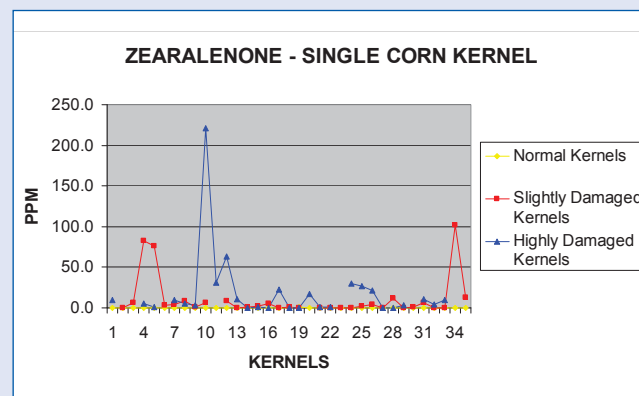
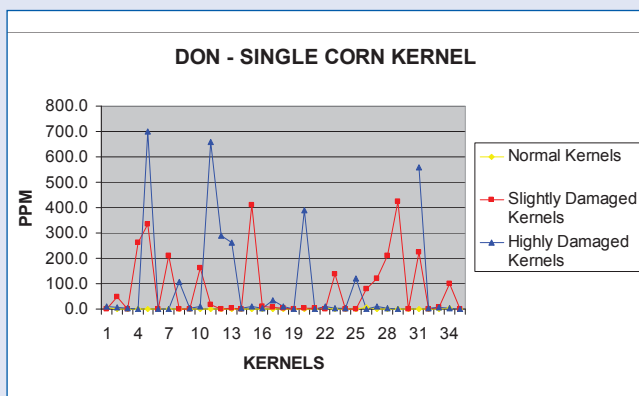


Group B - Slightly Damaged Kernels			
Kernel Weight(g)	DON ppm	Zearalenone ppm	Mean
1	15.34	0.44	15.34
2	49.16	0.23	49.16
3	263.17	82.84	263.17
4	335.34	76.13	335.34
5	1.95	3.40	1.95
6	211.31	3.87	211.31
7	2.00	4.49	2.00
8	0.41	1.16	0.41
9	163.62	6.37	163.62
10	16.29	0.17	16.29
11	0.61	8.72	0.61
12	0.43	2.94	0.43
13	0.58	0.59	0.58
14	419.90	1.71	419.90
15	10.66	5.87	10.66
16	4.78	0.00	4.78
17	1.85	0.07	1.85
18	0.89	0.00	0.89
19	3.27	0.00	3.27
20	3.13	0.00	3.13
21	0.00	0.00	0.00
22	0.00	0.00	0.00
23	138.38	0.17	138.38
24	0.32	0.11	0.32
25	0.73	1.61	0.73
26	80.94	4.19	80.94
27	125.10	0.00	125.10
28	299.39	11.36	299.39
29	424.15	0.00	424.15
30	0.41	1.18	0.41
31	224.17	5.99	224.17
32	0.69	0.00	0.69
33	0.47	0.46	0.47
34	100.91	100.29	100.91
35	0.00	22.78	0.00
Mean	0.34	79.76	10.83



Group C - Highly Damaged Kernels			
Kernel Weight(g)	DON ppm	Zearalenone ppm	Mean
1	0.55	15.68	0.55
2	0.34	8.40	0.34
3	0.16	4.19	0.16
4	0.37	0.32	0.37
5	0.23	699.32	0.23
6	0.23	0.50	0.23
7	0.23	0.00	0.23
8	104.64	5.32	104.64
9	1.92	3.73	1.92
10	0.18	221.55	0.18
11	0.27	659.00	0.27
12	0.18	286.76	0.18
13	0.24	291.37	0.24
14	0.24	1.77	0.24
15	0.19	11.79	0.19
16	0.22	2.96	0.22
17	0.34	33.19	0.34
18	0.22	8.92	0.22
19	0.23	0.08	0.23
20	0.22	389.09	0.22
21	0.24	0.18	0.24
22	0.28	0.79	0.28
23	0.19	5.13	0.19
24	0.28	174.30	0.28
25	0.27	120.40	0.27
26	0.19	0.48	0.19
27	0.29	5.24	0.29
28	0.24	2.83	0.24
29	0.31	0.00	0.31
30	0.14	559.03	0.14
31	0.18	2.11	0.18
32	0.16	4.43	0.16
33	0.23	8.99	0.23
34	0.10	1.97	0.10
35	0.16	0.60	0.16
Mean	0.23	94.82	18.33

Kernel Examples



Conclusions

- On average, the individual kernel weights decreased while the DON and zearalenone results increased with the amount of physical damage observed.
- A majority of the damaged corn kernels were contaminated with DON and zearalenone. There was a small number of normal appearing corn kernels that were contaminated with DON or zearalenone
 - Normal Kernel Group
 - DON – 34% positive (range 0.1 – 7.0 ppm)
 - Zearalenone – 6% positive (range 0.1 -0.9 ppm)
 - Slightly Damaged Kernel Group
 - DON – 91% positive (range 0.4 – 424.1 ppm)
 - Zearalenone – 76% positive (range 0.1 – 102.1 ppm)
 - Highly Damaged Kernel Group
 - DON – 94% positive (range 0.2 – 699.0 ppm)
 - Zearalenone – 96% (range 0.1 – 221.5 ppm)
- The number of kernels contaminated with both DON and zearalenone for the normal, slightly damaged, and highly damaged kernel groups were 0%, 69%, and 89% respectively.
- There was no correlation in the concentration of DON and zearalenone in single kernels for either group.
- The amount of contamination of DON and zearalenone in individual kernels can not be predicted by the amount of kernel damage.