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Original article

The use of near infrared transmittance kernel sorting technology to salvage high quality grain from grain downgraded due to *Fusarium* damage

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ABSTRACT

The mycotoxins associated with specific *Fusarium* fungal infections of grains are a threat to global food and feed security. These fungal infestations are referred to as *Fusarium* Head Blight (FHB) and lead to *Fusarium* Damaged Kernels (FDK). Incidence of FDK >0.25% will lower the grade, with a tolerance of 5% FDK for export feed grain. During infestation, the fungi can produce a variety of mycotoxins, the most common being deoxynivalenol (DON). *Fusarium* Damaged Kernels have been associated with reduced crude protein (CP), lowering nutritional, functional and grade value. New technology has been developed using Near Infrared Transmittance (NIT) spectra that estimate CP of individual kernels of wheat, barley and durum. Our objective is to evaluate the technology's capability to reduce FDK and DON of downgraded wheat and ability to salvage high quality safe kernels. In five FDK downgraded sources of wheat, the lowest 20% CP kernels had significantly increased FDK and DON with the high CP fractions having decreased FDK and DON, thousand kernel weights (TKW) and bushel weight (Bu). Strong positive correlations were observed between FDK and DON ($r = 0.90$); FDK and grade ($r = 0.62$) and DON and grade ($r = 0.62$). Negative correlations were observed between FDK and DON with CP ($r = -0.27$ and -0.32); TKW ($r = -0.45$ and -0.54) and Bu ($r = -0.79$ and -0.74). Results show improved quality and value of *Fusarium* downgraded grain using this technology.

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1. Introduction

Wheat is the 2nd most commonly grown grain next to maize and a top five commodity worldwide for use in food and feed (FAOSTAT, 2013). It is also susceptible to a fungal species, *Fusarium*, and responsible for *Fusarium* Head Blight (FHB). *Fusarium* infection usually occurs at the plant flowering stage (i.e. anthesis) and

incidence and severity is increasing worldwide (McMullen and Gallenberg, 1997). FHB causes physical damage to kernels and is referred to as *Fusarium* Damaged Kernels (FDK; Gilbert and Tekauz, 2000). The first sign of FHB is a change in kernel spikelet color from green to white in the grain head. These kernels are shrivelled, which is associated with disruption of kernel development (CAST, 2003). Although FHB infestation is most common in temperate regions, its occurrence has been identified in all regions. *Fusarium graminearum* is the most common *Fusarium* species infecting grains (Parry et al., 1995), and is the principle cause of FHB in Saskatchewan (Charmley and Trenholm, 2012), affecting the grain industry (Windels, 2000). Yield losses due to FDK are widespread (McMullen and Gallenberg, 1997; Bai et al., 2001) resulting in reductions in grain yield and quality, impacting the suitability for human and animal consumption (Dexter et al., 1996; Jin et al., 2014).

Although the level of FDK may not always be correlated to mycotoxin type or concentration (Liu et al., 1997), it does provide a

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rapid and economical means of reducing risk and grading grain. Testing large numbers of samples for a variety of mycotoxin contaminants is expensive and time consuming (Beyer et al., 2007). Common *Fusarium* species (i.e. *graminearum*, *culmorum*, *crookwellense*, and *verticilloides*) have adapted to a wide range of habitats (Moss, 1991). During kernel infestation crude protein (CP), an important quality and nutritional trait (Tonning et al., 2009), can be negatively affected (Matthaus et al., 2004; Paul et al., 2005). Reduction in CP may be due to the destruction of the protein that surrounds starch granules (Nightingale et al., 1999; Jackowiak et al., 2005); thereby lowering yield, milling and baking quality of flour (Dexter et al., 1996; Evers, 2000; Snape et al., 2005). It has been suggested that minimizing CP heterogeneity to produce more uniform CP levels within a batch of grain could lead to improved processing capabilities and overall grain quality (Benseler, 2010). This would also suggest that removal of low CP grains may significantly reduce levels of mycotoxins (D'Mello and Macdonald, 1998).

There is increasing evidence of global contamination of grains by *Fusarium* mycotoxins (D'Mello and Macdonald, 1998) with deoxynivalenol (DON) considered the most prevalent (Pronk et al., 2002). It has been established that DON is associated with FDK (Symons et al., 2002) and indications are that removal of FDK would reduce DON, and thereby increase grain quality and safety. Health Canada has established maximum allowable levels of DON in grain for human consumption to be 1.0 ppm (Health Canada, 2012). Grain grading quality standards are used to subjectively evaluate grain quality for nutritional and functional value as food or feed (Alander et al., 2013). In Canada, key determinants to identify top quality grain (classified as No. 1 grade) from downgraded grain are the factors relating to level of CP and mycotoxins. Nearly 50 visual factors of grain quality are used in determining grade with grain that contains a higher level of FDK, and potentially higher levels of mycotoxins. If FDK is >4% the grain will be limited to feed usage and >6% FDK is graded as "Salvage". Estimates for the 2014 crop year are that 2 MMT (million metric tonnes) of wheat and durum were graded as Salvage primarily due to FDK (Dr. Rex Newkirk, Canadian International Grain Commission, personal communication, 2014). Typically grain graded as Feed has approximately 30% less value than grain graded as No. 1 and it is illegal to market Salvage grain. It would normally be disposed of or diluted with higher quality grain to meet minimum standards for sale.

The relationship between visual grading for FDK and actual levels of DON are not consistent (Peiris et al., 2010) due to DON variation within kernels (Beyer et al., 2007, 2010; Berthiller et al., 2013) but it is generally accepted as the only rapid and economic means of grading grain for safety. Beyer et al. (2010) evaluated the effectiveness of Near Infrared (NIR) spectrometry to differentiate healthy, sound kernels from FDK. They concluded that the largest spectral changes between healthy and damaged kernels occurred in the 1400 and 1900 nm spectral range. Peiris et al. (2009) observed that absorption bands of DON were in a similar spectral range, indicating that DON level could be estimated from specific NIR spectra. A single kernel NIR (SKNIR) system was developed by Perten Instruments (Stockholm, Sweden) to detect FDK accurately (Peiris et al., 2010) but was unable to sort large volumes of contaminated grain. BoMill AB (Lund, Sweden) has engineered a Near Infrared Transmittance (NIT) sorter, the TriQ (Fig. 1) that has higher sorting capacity and uses a limited algorithm of spectra. The use of NIT spectroscopy allows a single kernel to be evaluated for internal CP. Since FHB produces FDK and these kernels may contain reduced CP, the TriQ was evaluated to determine if it was capable of accurately reducing FDK and by association DON by sorting individual kernels based on CP.

Our objective was to evaluate this technology's capability for salvaging grain from multiple sources of downgraded due to

Fusarium infection (Canadian Grain Commission, 2013). A capacity to reduce FDK and DON and salvage high quality grain would significantly reduce risks for humans and animals and increase food and feed security. The authors' note that such sorting technology would change the view of grain as a mass commodity to one based on individual kernels and has significant ramifications for food, malt and feed production.

2. Materials and methods

2.1. BoMill TriQ sorter

The development of the patented TriQ (BoMill AB, 2008; Patent #7417203) individual kernel sorter was based on application of NIT technology. This technology measures and sorts individual kernels of wheat, barley or durum based on variability in the spectral range of 1100–1700 nm, which is used to estimate CP. The spectra of individual kernels with known CP were used to establish reference values for the TriQ to determine the CP of unknown kernels.

The TriQ uses a stainless steel rotating drum (i.e. singulator) that has 256 rows of 88 laser etched singulator pockets designed to position individual kernels and allow near infrared wavelengths to pass through the kernels. Specifically designed drums are required for each grain type, and at present drums have been designed to sort wheat, durum and barley. The rotating drum uses centrifugal force to position individual kernels into the singulator pockets and carries the kernels past three detectors. These detectors measure NIT wavelengths passing through the kernels and obtain six to ten readings per kernel that are used to determine where the grain will be ejected. The processed information is then relayed to the compressed air ejection unit that ejects individual kernels into one of three user determined ejection outlets. A single unit can sort grain at a rate of 2–3 tonnes/h.

Prior to commercial sorting, the drums require calibration. Calibration is based on scanning a sample (~100–200 kg) of grain within the TriQ using a specified number of near infrared spectra to determine the variability in kernel CP. Once the calibration curve has been established the TriQ software will produce a histogram indicating ten equal fractions of grain based on individual kernel CP. These ten fractions are then produced for sampling in sequence from two of the three ejection outlets: one for the designated calibration fraction (~3–5 kg) and one for the remaining grain. These samples can be analyzed to verify the actual variability in CP (and indirectly FDK and DON) and grade; then the user is able to define three commercial fractions for sorting a sample of grain. The focus of this work is to discuss the ten calibration fractions and assess this variability from five different wheat sources. During detection some kernels are not properly identified and are classified as outliers. The outliers may represent kernels from other types of grain, improper positioning of kernels into pockets or two kernels in one pocket (BoMill, 2012). These outliers are included in the first and last of the ten calibration fractions. Typically outliers are reduced by pre-cleaning grain and removing excessively small seeds; this was not done in the present study. In future studies we will use the three commercial fractions to identify three components of the bulk grain: kernels that are classified as outliers, kernels containing the two lowest calibration fractions representing 20% of the kernels having the lowest CP as the second commercial fraction; and the remaining grain from the eight calibration fractions as the third commercial fraction. This amounts to approximately 400 kg as the lowest 20% of the kernels on a 2 MT sort basis. The commercial fractions will be used to determine grade and level of DON to assess the capacity to salvage high quality grain from downgraded grain.

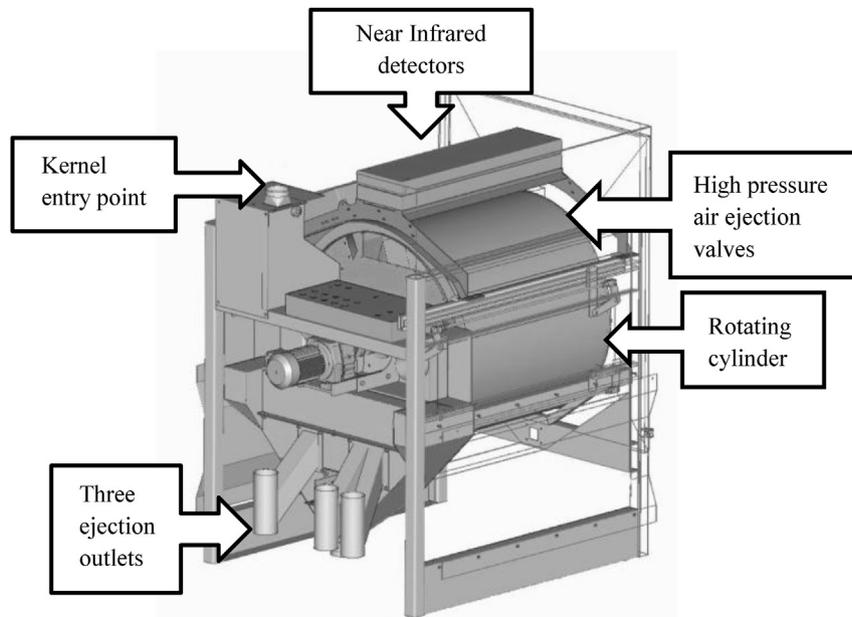


Fig. 1. TriQ manufactured by BoMill. Note the location of the near infrared detectors at the top of the figure and the three ejection outlets at the bottom left. The TriQ dimensions (height × width × depth) are 1.8 m × 1.2 m × 1.75 m. Picture rendition from BoMill (www.bomill.com), 2014.

2.2. Wheat sources

Five sources of wheat (6 MT/source) of grain downgraded based primarily on levels of FDK were purchased from grain producers in Western Canada. The characteristics of each grain source were defined in Table 1. The five wheat samples were classified as: Canadian Western Red Spring (CWRS; $n = 2$); Canadian Western Soft White Spring (CWSWS; $n = 2$); and Canadian Western Amber Durum (CWAD; $n = 1$). Representative samples of 750 g of each grain were graded by a Canadian Grain Commission inspector. The grade information included: grade; bulk density (kg/hL); and FDK (%) as defined by Official Grain Grading Guide (Canadian Grain Commission, 2013). Samples were collected from each of the sorted ten calibration fractions generated (2 kg) and from each unsorted grain (1 kg) for characterization and mycotoxin analysis. For the calibration sort and subsequent analysis, outlier grain was included in the lowest 10% and highest 10% CP fractions.

2.3. Chemical assessments

All grain samples were ground through a 1 mm screen (SM 2000 High-Performance Cutting Mill, Retsch GmbH and Co., Haan, Germany) and thoroughly mixed prior to analysis. Ground samples

(0.1 g) were analyzed in duplicate for CP ($N \times 6.25$) with a Leco nitrogen analyzer (model FP528 601-500-100; Leco Corp., St. Joseph, MI, USA) using 0.1 g EDTA (ethylenediaminetetraacetic acid) as a standard (AOAC, 1995). Crude protein was evaluated on a dry matter (DM) basis using the formula $CP (DM) = CP \%/[(100 - \text{moisture } \%/100)]$.

2.4. Mycotoxin determinations

Mycotoxin analysis was conducted at North Dakota State University (Veterinary Diagnostic Laboratory, Fargo, North Dakota) based on their proprietary protocol. The suite of 16 mycotoxins measured included: deoxynivalenol (DON or vomitoxin) and metabolites (3 and 15 acetyl DON); T-2 toxin and metabolites (T-2 triol, T-2 tetraol, Iso T-2 toxin and acetyl T-2); HT-2 toxin; fusarenone-X (FUS); diacetoxyscirpenol (DAS); scirpentriol (SCR) and metabolite (15 acetyl scirpentriol); nivalenol (NIV); neosolaniol (NEO); zearalenol (ZEL); and zearalenone (ZEN). A standard curve using 0.2, 0.5, 1.0, 3.0 and 6.0 ppm *Fusarium* mycotoxins standards (Romer Labs Diagnostic GmbH, Tulln, Austria) mixed in a known wheat blank (0.0 ppm *Fusarium* mycotoxins) was prepared. A “wheat pool” sample produced by the lab was used as a positive control. Samples of 25 g were prepared in duplicate and analyzed through a solvent extraction solution of acetonitrile and water. Chemical derivatization was used to enhance the volatilization of potential mycotoxins for more accurate detection. The solution was filtered using a 1:1 mixture of C_{18} and alumina. Samples were analyzed using gas chromatography (model 6890N; Agilent Technology, Englewood, CO, USA) and mass spectrometer (model 5975B XL E1/C1; Agilent Technology, Englewood, CO, USA). Mirex (Absolute Standards Inc., Hamden, CT) was dissolved in iso-octane to establish a quantitation peak and all mycotoxin concentrations were determined using linear regression. Of all mycotoxins tested, only DON was found to be above the detection limit (0.5 ppm) in any of the grain samples.

2.5. Physical assessment – grading

Samples of unsorted grain and ten calibration fractions were graded by inspectors of the Canadian Grain Commission and

Table 1

The estimations of *Fusarium* Damaged Kernels (FDK), deoxynivalenol (DON), grade, crude protein (CP), thousand kernel weight (TKW) and bushel weight (Bu) of four sources of wheat (CWRS and CWSWS) and one source of durum wheat (CWAD) downgraded due to FDK.

Wheat types	FDK, %	DON, ppm	Grade ^d	CP, %	TKW, g	Bu, kg/hL
CWRS ^a 01	1.4	1.1	3	19.7	38.5	73.7
CWRS ^a 02	1.6	1	4	19.7	37.7	74.4
CWSWS ^b 01	1.7	0.9	4	14.5	33.2	75.8
CWSWS ^b 02	3.7	1.2	4	16.3	42.4	76.9
CWAD ^c	7.6	8.4	6	18.1	43.7	74.1

^a CWRS = Canadian Western Red Spring.

^b CWSWS = Canadian Western Soft White Spring.

^c CWAD = Canadian Western Amber Durum.

^d Grade (based on Canadian grading classifications from 1 to 6 where 4 = Feed and 6 = Salvage).

provided legal grade and estimates of %FDK. Thousand kernel weight (TKW) (TKW, g) from each calibration fraction were determined in triplicate using an ESC-1 seed counter (model ESC120006; Agriculex Inc., Guelph, ON, CAN) and bushel weight (Bu) (Bu, kilogram per hectoliter – kg/hL) was determined in triplicate with a UK imperial pint based on the formula: $Bu = (kg/pint)/(pint/hL)$, where $pint/hL = 0.00568$.

2.6. Calculations

The data on grain quality was highly variable due to grain source and this interfered with our ability to correlate the differences between calibration fractions across all grain sources. Therefore, we expressed the values in fractions 2 to 10 relative to the first fraction and then calculated the correlations for the different measurements (i.e., CP, FDK, DON, TKW and Bu).

2.7. Statistical design

The variables described above for the ten calibration fractions were analyzed using a complete randomized design (PROC MIXED of SAS [Version 9.3]; SAS Institute, Cary, NC) statistical model ($y_{ij} = \mu + \tau_i + \varepsilon_{ij}$) where: y = measured parameter; μ = overall mean; τ_i = fixed effects (individual calibration fractions) and ε_{ij} = error term for the fixed effects and associated variation between the five sources of wheat included. Multi-treatment means comparisons of the calibration fractions were adjusted using the Tukey test. CP, TKW and Bu analysis was based on the % difference compared to the first fraction. Linear associations between calibration fractions and all parameters were analyzed using Pearson (r) correlation coefficients [PROC CORR of SAS (Version 9.3); SAS Institute, Cary, NC] statistical model. Further analysis using linear regression [PROC REG of SAS (Version 9.3); SAS Institute, Cary, NC] model ($y_i = \alpha + \beta x_i + \varepsilon_i$, where α = y -intercept, β = slope of the line and ε_i = error term) to evaluate the relationship between the variables was conducted where appropriate. Data is presented as means with standard error of the mean (SEM). Significance was determined at $P < 0.05$.

3. Results

3.1. Unsorted grain

Descriptive data for the original five unsorted sources of grain are provided (Table 1). Each unsorted source was analyzed for FDK, DON, CP, TKW and Bu. The FDK of the five wheat sources ranged from 1.4 to 7.6%, with CWAD containing the highest level. For DON, the range was from 0.9 to 8.4 ppm, with CWAD containing the highest level. CP on a dry matter basis ranged from 14.5 to 19.7%, TKW from 33.2 (CWSWS 01) to 43.7 g (CWAD) and Bu from 73.9 (CWRS 01) to 76.9 kg/hL (CWSWS 02). Due to grading criteria being impacted by FDK, only the CWRS 01 sample graded #3. The CWRS 02 and both CWSWS wheat samples graded as Feed (#4) with CWAD graded as Salvage (#6) and not legal for sale. As Feed, AC Fusarium and commercial salvage grades are non-numerical, values of 4, 5 and 6 respectively were dedicated to Feed, AC Fusarium and salvage for statistical purposes.

3.2. Grain sorted into calibration fractions

Each unsorted source was sorted into the ten calibration fractions (10% increments of total variation; outliers were included in fractions 1 and 10) by the TriQ based on CP. Similar methods were used for all grain sources. Four wheat sources (CWRS and CWSWS) were sorted using the specifically designed wheat singulator; and a

different singulator was used for the durum (CWAD). To evaluate the sorters capacity to produce repeatable calibration fractions regardless of wheat type, data from the respective fractions for all sources of grain were pooled for statistical comparison (Table 2). There were significant differences between the ten calibration fractions for all measurements. Calibration fractions one and two were the most significantly different from all other fractions after sorting. In comparison to the original total unsorted grain (Table 2); these two fractions contained the highest level of FDK and DON while remaining fractions (3 to 10) indicated an improvement in all measurements. Grade for the higher fractions (3–10) was improved to either No. 1 or No. 2 (with a grade of Feed being the original assessment) with the exception of those fractions from the sorted amber durum (CWAD) where a grade of No. 3 was obtained (original assessment was Salvage). Part of the explanation for the durum was the higher contamination and damage due to FDK. Grain was also downgraded based on other characteristics not related directly to FDK; however, improvements in grade were observed.

Pearson correlations (Table 3) based on the individual calibration fractions indicated that all correlations were significant ($P < 0.01$). There was a strong positive correlation ($r = 0.90$) between FDK and DON. CP was negatively correlated with FDK ($r = -0.27$); DON ($r = -0.32$) and grade ($r = -0.51$) but positively correlated with both TKW ($r = 0.51$) and Bu ($r = 0.23$). It should be noted that the negative correlation with grade is actually an increase in grain value as decreasing numerical value indicates an improvement in grain quality. Grade was positively correlated with FDK ($r = 0.62$) and DON ($r = 0.62$) but negatively with both TKW ($r = -0.48$) and Bu ($r = -0.36$). There were negative correlations between TKW and FDK ($r = -0.45$) and DON ($r = -0.54$); but a positive correlation with Bu ($r = 0.31$). Finally Bu was negatively correlated with FDK ($r = -0.79$) and DON ($r = -0.74$). Regression analysis of FDK (y) and DON (x) indicated that approximately 80% of DON levels can be explained by FDK % ($y = 1.665 + 0.993x$, $P < 0.01$; $r^2 = 0.803$).

4. Discussion and conclusions

Previously, the ability to sort bulk grain based on individual kernel CP into user-defined fractions was unfeasible on a commercial scale. In the TriQ patent by Lofqvist and Nielsen (2003) the calibration and development of this technology was based on the results of sorting using NIT spectra and comparing the results to the chemically determined CP through Kjeldahl analysis. The use of NIT allows individual kernel internal structures such as CP to be evaluated based on spectral information being converted into proprietary algorithms as an indication of kernel internal structural integrity and soundness. From the results of this study, the BoMill TriQ sorter has demonstrated the capability to sort bulk sources of downgraded grain based on FDK indirectly by sorting directly on kernel CP. Reduction in FDK resulted in an associated reduction in DON and increased CP as indicated by the correlation results between FDK, DON and CP and past literature (reviewed by Paul et al., 2005; Beyer et al., 2007; Wegulo et al., 2010). We acknowledge that FDK has been reported without DON and vice versa (Mesterhazy, 2002). More sources of FDK downgraded grain are required to verify the consistency and capacity of the TriQ sorter.

As demonstrated previously by Tonning et al. (2009) and Benseler (2010), the TriQ is capable of differentiating individual kernels of grain based on CP however neither researcher indicated the effect of sorting by CP on FDK and associated mycotoxins. In the present study, the two lowest CP fractions contained increased FDK and DON while remaining fractions showed improved quality and value with greatest improvements to CP seen in fractions eight to

Table 2

The assessment of *Fusarium* Damaged Kernels (FDK), deoxynivalenol (DON), grade, crude protein (CP), thousand kernel weight (TKW) and bushel weight (Bu) for the ten calibration fractions produced by the BoMill TriQ individual kernel sorter representing variation in four sources of wheat and one source of durum wheat.

	<i>n</i> ^b	FDK, %	DON, ppm	Grade	CP, %	TKW, g	Bu, kg/hL
Unsorted Combined average		2.44	1.67	2.90	18.1	40.4	76.7
Fractions		**	**	**	**	**	**
1 ^a	10	9.79a	7.38a	5.60a	17.7c	38.3c	72.8h
2	10	5.65ab	2.78b	4.60ab	17.8bc	39.3bc	74.9g
3	10	2.51b	1.62b	3.60abc	17.9abc	41.0a	75.8fg
4	10	1.67b	1.10b	2.60bc	18.1ab	41.4a	76.3ef
5	10	2.09b	0.88b	2.40bc	18.2ab	40.8a	76.8de
6	10	0.61b	0.80b	2.00c	18.2ab	41.1a	77.4cd
7	10	1.03b	0.64b	2.20bc	18.3a	41.6a	77.8bc
8	10	0.48b	0.54b	2.00c	18.3a	41.2a	78.7ab
9	10	0.43b	0.48b	2.20bc	18.1ab	40.7ab	79.3a
10 ^a	10	0.13b	0.52b	1.80c	18.0abc	39.0c	77.1cde
SEM	100	0.542	0.280	0.207	0.235	0.314	0.221

Means with different letters in the same column are significantly different. Significance indicated as: NS ($P \geq 0.1$); † ($P \leq 0.1$); * ($P \leq 0.05$); ** ($P \leq 0.01$) and *** ($P \leq 0.001$).

^a Outliers were not removed prior to analysis.

^b Number of measurements per fraction.

Table 3

Pearson correlation coefficients between the measured variables (based on sorting by crude protein) of *Fusarium* Damaged Kernels (FDK), deoxynivalenol (DON), grade, crude protein (CP), thousand kernel weight (TKW) and bushel weight (Bu) from all calibration fractions ($n = 100$) of all tested grain sources are compared statistically.

Item	FDK, %	DON, ppm	CP, %	Grade	TKW, g	Bu, kg/hL
DON, ppm	0.90***	1.00				
CP, %	-(0.27)**	-(0.32)**	1.00			
Grade	0.62***	0.62***	-(0.51)***	1.00		
TKW, g	-(0.45)***	-(0.54)***	0.51***	-(0.48)***	1.00	
Bu, kg/hL	-(0.79)***	-(0.74)***	0.23**	-(0.36)***	0.31**	1.00

Significance indicated as: NS ($P \geq 0.1$); † ($P \leq 0.1$); * ($P \leq 0.05$); ** ($P \leq 0.01$) and *** ($P \leq 0.001$).

Correlations are calculated using the differences in numerical values in proportion to fraction 1 to account for variability of different wheat sources.

ten. Visual grading by a Canadian Grain Commission inspector of each of the ten calibration fractions from all sources demonstrated that grade was significantly improved when FDK was removed. The decreased CP in fractions one and ten may be associated with the outlier kernels being automatically assigned to these fractions in the calibration sort. It has been suggested that pre-cleaning bulk grain would reduce the number of outlier kernels and minimize the impacts on fraction ten (Bo Lofqvist, 2014 personal communication).

The TriQ does not sort solely on CP. The selected spectral ranges utilized by the TriQ are also correlated to other indicators (i.e. hardness, vitreousness; Bo Lofqvist, 2014 personal communication). These correlations allow spectra from unknown grain to be compared against known spectra for the determination of CP in grain sources. A potential reason for the lack of more pronounced CP variability within the ten calibration fractions could be the limited variability within the bulk grain obtained. Further work is also required to establish the sensitivity and accuracy of sorting grain with higher variability in individual kernel CP.

Downgrading of grain is typically in response to a small percentage of affected kernels that reduce the safety or quality. This study has focused on the merits and capacity of removing these low quality kernels and salvaging the high quality kernels. With the increasing awareness of food security issues and one third of food being lost or wasted (Gustavsson et al., 2011), salvage of the high quality kernels for use in food production is vital (Benseler, 2010). Decrease in grain grade can occur from as little as 0.25% FDK (CWRS) to 1.5% FDK (CWSWS) (Canadian Grain Commission, 2013). Decreased levels of FDK and DON had a positive impact on the correlation to grade ($r = 0.62$).

The relationship between TKW and fraction observed in the present study was different than reported by Benseler (2010).

While Benseler indicated that TKW decreased with increasing fraction, our results indicated a moderate increase and plateau. Analysis of Bu was in agreement showing a linear increase with increasing fraction. These measurements of TKW and Bu weight can be influenced by a number of factors including kernel packing, size and density (Nielsen et al., 2003).

The relatively small size of FDK compared to uninfected kernels affects TKW and Bu weight, as indicated by the negative correlations. This could lead to suggestions that the TriQ potentially sorts on kernel size. However, results by Tonning et al. (2009) indicated that the TriQ did not sort based on kernel size through observations of kernel mass, diameter and hardness measurements. Additionally, Benseler (2010) saw no significant differences in weight or size with barley using three screen sizes to obtain four size fractions. Most kernels were located in a similar screen range indicating similar sizes except fractions eight to ten which showed a decrease in size with the smallest kernels located in fraction ten and may be actually outliers that are assigned to fraction ten. *Fusarium* infected kernels tend to be smaller, lighter and shrunken leading to reduced weight (Matthaus et al., 2004; Jin et al., 2014).

This study indicates that the TriQ has the potential to sort bulk grain sources based on CP and reduce FDK and DON. Since FDK have been strongly correlated with mycotoxins like DON, their detection in grain sources is an issue of food safety (Beyer et al., 2010). If these damaged kernels could be safely removed from grains, it would improve grain quality through reduction in variability; turn low quality ingredients into high quality food or feed; increase milling performance and baking with consistently better grain quality; decrease feed waste (which accounts for 60–70% of feed value); and reduce in the spread of FHB by improving kernel stock (Gilbert and Tekauz, 2000). By removing as little as 20% of the low CP kernels, grain quality can be improved and previously unusable grain

in terms of regulatory restrictions can be salvaged for human or animal consumption. Additional capabilities include the recombination of these ten calibration fractions into a commercial sort of three fractions: one to concentrate FDK and DON for removal; a second for outliers, preventing those kernels from impacting a third fraction consisting of kernels with improved safety and security. Upgrades to the TriQ software providing the ability to sort bulk grain specifically on *Fusarium* damaged kernels or hard vitreous kernels will improve the sorter capabilities to remove healthy from infected kernels. With many countries setting regulatory limits for mycotoxins in foods and feeds (van Egmond, 2004, van Egmond et al., 2007) and the persistence and effects of mycotoxins continuing to affect global communities after 30 years of research (Cardwell et al., 2001) agricultural technologies such as the BoMill TriQ may provide an effective solution for the reduction or removal of FDK and associated mycotoxins.

Conflict of interest statement

The authors declare no competing financial interest.

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References

- AOAC. Official methods of analysis. 16th ed. Arlington: Virginia; 1995.
- Alander JT, Bochko V, Martinkauppi B, Saranwong S, Mantere T. A review of optical nondestructive visual and near-infrared methods for food quality and safety. *Inter J Spect* 2013;1–36. Article ID 341402.
- Bai GH, Plattner R, Desjardins A, Kolb F. Resistance to *Fusarium* head blight and deoxynivalenol accumulation in wheat. *Plant Breed* 2001;120:1–6.
- Benseler C. Evaluation of new technologies for the fractionation of cereal lots by single particle analysis. Unpublished Thesis Dissertation. Freising: Science Centre Weihenstephan, Technical University of Munich; 2010.
- Berthiller F, Crews C, Dall'Asta C, De Saeger S, Haesaert G, Karlovsky P. Masked mycotoxins: a review. *J Mol Nutr Food Res* 2013;57(1):165–86.
- Beyer M, Klix MB, Verreet JA. Estimating mycotoxin contents of *Fusarium* damaged winter wheat kernels. *Int J Food Microbiol* 2007;119(3):153–8.
- Beyer M, Pogoda F, Ronellenfitsch FK, Hoffmann L, Udelhoven T. Estimating deoxynivalenol contents of wheat samples containing different levels of *Fusarium* damaged kernels by diffuse reflectance spectrometry and partial least square regression. *Int J Food Microbiol* 2010;142:370–4.
- BoMill AB. Hardware and software user manual. 2012. TriQ30. Version 1.1.
- Canadian Grain Commission. Primary grade determinants tables. Wheat – Chapter 4. Guides and manuals. 2013. <https://www.grainscanada.gc.ca/oggg-gocg/04/oggg-gocg-4e-eng.htm> [accessed 05.10.14].
- Cardwell KF, Desjardins A, Henry SH, Munkvold G, Robens J. Mycotoxins: the cost of achieving food security and quality. APSNET; 2001.
- Center for Applied Special Technology (CAST). Mycotoxins: risks in plant, animal, and human systems. Council for agricultural Science and technology Task force. Ames: Iowa; 2003.
- Charmley LL, Trenholm HL. Fact sheet – mycotoxins. RG-8 regulatory Guidance: Contaminates in feed. Section 1. Mycotoxins in livestock feed. Canadian Food Inspection Agency; 2012. <http://www.inspection.gc.ca/animals/feeds/regulatory-guidance/rg-8/eng/1347383943203/1347384015909?chap=1> [accessed 03.10.14].
- Dexter JE, Clear RM, Preston KR. *Fusarium* head blight: effect on the milling and baking of some Canadian wheats. *Cereal Chem* 1996;73:695–701.
- D'Mello JPF, Macdonald AMC. Fungal toxins as disease elicitors. In: Rose J, editor. Environmental toxicology – current developments, United Kingdom. Gordon and Breach Science Publishers; 1998. p. 253–89.
- Evers AD. Grain size and morphology: implications for quality. In: Schofield D, editor. Wheat structure, biochemistry and functionality. United Kingdom: Royal Society of Chemistry; 2000. p. 19–24.
- FAOSTAT. Food and Agriculture Organization of the United Nations. 2013. <http://faostat3.fao.org/browse/Q/QC/E> [Accessed 15.01.15].
- Gilbert J, Tekauz A. Review: recent developments in research on *Fusarium* head blight of wheat in Canada. *Can J Plant Pathol* 2000;22:1–8.
- Gustavsson J, Cederberg C, Sonesson U, van Otterdijk R, Meybeck A. Global food losses and food waste: extent, causes and prevention. Rome: Food and Agriculture Organisation of the United Nations (FAO); 2011. p. 29.
- Health Canada. Canadian standards (maximum levels) for various chemical contaminants in foods. Food safety, chemical contaminants. 2012. <http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/contaminants-guidelines-directives-eng.php> [accessed 05.10.14].
- Jackowiak H, Packa D, Wiwart M, Perkowski J. Scanning electron microscopy of *Fusarium* damaged kernels of spring wheat. *Inter J Food Microbiol* 2005;98:113–23.
- Jin F, Bai G, Zhang D, Dong Y, Ma L, Bockus W. *Fusarium* damaged kernels and deoxynivalenol in *Fusarium* infected U.S. winter wheat. *Phytopathology* 2014;104(5):472–8.
- Liu W, Langseth W, Skinnis H, Elen ON, Sundheim L. Comparison of visual head blight ratings, seed infection levels, and deoxynivalenol production for assessment of resistance in cereals inoculated with *Fusarium culmorum*. *Eur J Plant Pathol* 1997;103:589–95.
- Lofqvist B, Nielsen JP. A method for sorting objects comprising of organic material. Patent WO 03/004179 A1, 2003.
- Matthaus K, Danicke S, Vahjen W, Simon O, Wang J, Valenta H. Progression of mycotoxin and nutrient concentrations in wheat after inoculation with *Fusarium culmorum*. *Arch Anim Nutr* 2004;58:19–35.
- McMullen M, Gallenberg D. Scab of wheat and barley: a re-emerging disease of devastating impact. *Plant Dis* 1997;81(12):1340–8.
- Mesterhazy A. Role of deoxynivalenol in aggressiveness of *Fusarium graminearum* and *F. culmorum* and in resistance to *Fusarium* head blight. *Eur J Plant Pathol* 2002;108:675–84.
- Moss MO. Mycology of cereal grain and grain products. In: Chelkowski J, editor. Cereal grain: mycotoxins, fungi and quality in drying and storage. New York: Elsevier Science Publishing Inc; 1991. p. 23–51.
- Nielsen JP, Pederson DK, Munck L. Development of non-destructive screening methods for single kernel characterization of wheat. *Cereal Chem* 2003;80(3):274–80.
- Nightingale MJ, Marchylo BA, Clear RM, Dexter JE, Preston KR. *Fusarium* head blight: effect of fungal proteases on wheat storage proteins. *Cereal Chem* 1999;76:150–8.
- Parry DW, Jenkinson P, McLeod L. *Fusarium* ear blight (scab) in small grain cereals – a review. *Plant Pathol* 1995;44(2):207–38.
- Paul PA, Lipps PE, Madden LV. Relationship between visual estimates of *Fusarium* head blight intensity and deoxynivalenol accumulation in harvested wheat grain: a meta-analysis. *Phytopathology* 2005;95(10):1225–36.
- Peiris KHS, Pumphrey MO, Dowell FE. NIR absorbance characteristics of deoxynivalenol and of sound and *Fusarium* damaged wheat kernels. *J Near infrared Spectrosc* 2009;17:213–21.
- Peiris KHS, Pumphrey MO, Dong Y, Maghirang EB, Berzonsky W, Dowell FE. Near-infrared spectroscopic method for identification of *Fusarium* head blight damage and prediction of deoxynivalenol in single wheat kernels. *Cereal Chem* 2010;87:511–7.
- Pronk MEJ, Schothorst RC, van Egmond HPV. Toxicology and occurrence of nivale-nol, fusarenon X, diacetoxyscirpenol, neosolaniol and 3- and 15- acetyldeoxynivalenol; a review of six trichothecenes. 2002. RIVM Report 388802024. Netherlands: Utrecht.
- Snape J, Fish L, Leader D, Bradburne R, Turner A. The impact of genomics and genetics on wheat quality improvement. *Turk J Agric Forum* 2005;29:97–103.
- Symons SJ, Clear RM, Bell K, Butler C. Identifying wheat and barley seed affected by *Fusarium* head blight. Grain biology bulletin No.2. 3rd ed. Winnipeg: Canada: Grain Research Laboratory, Canadian Grain Commission; 2002.
- Tonning E, Thybo AK, Pederson L, Munck L, Hansen A, Togersen FA. Bulk functionality diversification by unsupervised single kernel near infrared (SKNIR) sorting of wheat. *Cereal Chem* 2009;86:706–13.
- Van Egmond HP. Natural toxins: risks, regulations and the analytical situation in Europe. *Anal Bioanal Chem* 2004;378:1152–60.
- Van Egmond HP, Schothorst RC, Jonker MA. Regulations relating to mycotoxins in food. *Anal Bioanal Chem* 2007;389:147–57.
- Wegulo SN, Bockus WW, Nopsa JH, De Wolf ED, Eskridge KM, Peiris KHS. Effects of integrating cultivar resistance and fungicide application on *Fusarium* head blight and deoxynivalenol in winter wheat. *Plant Dis* 2010;95:554–60.
- Windels CE. Economic and social impacts of *Fusarium* head blight: changing farms and rural communities in the Northern Great Plains. *Phytopathology* 2000;90(1):17–21.