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Rapid and non-invasive analysis of deoxynivalenol in durum and common wheat by Fourier-Transform Infrared (FT-NIR) spectroscopy

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Running title: FT-NIR analysis of deoxynivalenol in wheat

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Abstract

Fourier transform near-infrared spectroscopy (FT-NIR) was used for rapid and non-invasive analysis of deoxynivalenol (DON) in durum and common wheat. The relevance of using ground wheat samples with a homogeneous particle size distribution to minimize the variation of measurements and avoid DON segregation among particles of different sizes was established. Calibration models for durum wheat, common wheat and durum + common wheat samples, with particle size < 500 µm, were obtained by using Partial Least Squares (PLS) regression with the external validation technique. Values of root mean square error of prediction (RMSEP, 306-379 µg kg^{-1}) were comparable and not too far from values of root mean square error of cross-validation (RMSECV, 470-555 µg kg^{-1}). Coefficients of determination (r^2) indicated an "approximate to good" level of prediction of the DON content by FT-NIR spectroscopy in the PLS calibration models (r^2 = 0.71-0.83), and a "good" discrimination between low and high DON contents in the PLS validation models (r^2 = 0.58-0.63). A "limited to good" practical utility of the models was ascertained by range error ratio (RER) values higher than 6. A qualitative model, based on 197 calibration samples, was developed to discriminate between blank and naturally contaminated wheat samples by setting a cut off at 300 µg kg^{-1} DON to separate the two classes. The model correctly classified 69% of the 65 validation samples with most misclassified samples (16 out of 20) showing DON contamination levels quite close to the cut off level. These findings suggest that FT-NIR analysis is suitable for the determination of DON in unprocessed wheat at levels far below the DON maximum permitted limits set by the European Commission.

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Keywords: FT-NIR spectroscopy; deoxynivalenol; wheat; PLS regression, discriminant analysis.

Introduction

Deoxynivalenol (DON) is a type B trichothecene mycotoxin produced by fungi of the *Fusarium* genus, in particular *Fusarium graminearum* (Gibberella zea) and *Fusarium culmorum.* It is frequently associated with contaminated cereal crops such as wheat, maize, barley, oats, rye and less often with rice, sorghum and triticale (Canady et al. 2001; Schothorst and van Egmond 2004). DON inhibits the synthesis of DNA, RNA and proteins, and has a hemolytic effect on erythrocytes. In animals, acute/subacute oral toxicity of DON is characterized by vomiting, feed refusal, weight loss and diarrhea (Schlatter 2004).

Based on the temporary tolerable daily intake (TDI) of 1 µg/kg body weight established by the EU Scientific Committee on Food (SCF) (SCF 2002), the European Commission has set maximum permitted levels for DON in a variety of cereal based foods (European Commission 2007). In particular, 1750 µg kg^{-1} and 1250 µg kg^{-1} were set as maximum levels for unprocessed durum and common wheat, respectively; lower levels were set for wheat flour, bran, germ and pasta (750 µg kg^{-1}), for wheat and wheat products for direct human consumption (750 µg kg^{-1} and 500 µg kg^{-1}), and for wheat-based infants and baby food (200 µg kg^{-1}).

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Rapid and sensitive methods for the quantification of DON in wheat and wheat-based food products are strongly demanded by private and public quality control laboratories, as well as in surveys aiming to provide data for risk assessments. Several methods based on competitive enzyme-linked immunosorbent assays (ELISA) have been developed for the rapid screening and quantification of DON in cereals. ELISA methods are sensitive, rapid and easy-to-use, but they often show strong cross-reactivity against DON acetyl derivatives, require long incubation times for complete antigen-antibody reaction and commercial kits are still rather expensive. A fluorescence polarization (FP) immunoassay has been recently developed for the rapid and quantitative analysis of DON in durum wheat, semolina, and pasta at levels below the EU maximum permitted levels (Lippolis et al. 2006).

Although these methods can be used for screening purposes, they are destructive and require preparation and extraction steps. In the past few decades, many researchers have focused on the potential use of infrared spectroscopy in the analysis of food and feed, both in the mid- and near-infrared regions. Infrared spectroscopy (IR) is a rapid analytical technique requiring very little labour, once a calibration is obtained, and no sample extraction and, therefore, does not create chemical waste.

Petterson and Aberg (2003) used near infrared (NIR) transmittance for the determination of DON in whole wheat kernel samples at levels higher than 500 μg kg^{-1}, whereas NIR reflectance has been used for detection of scab and estimation of DON and ergosterol in single kernels of highly infected wheat (Dowell et al. 1999). A method for the analysis of

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Fusarium fungi on corn using Fourier transform-mid infrared (FT-MIR) spectroscopy with attenuated total reflection (ATR) was developed which enabled the segregation of DON contaminated corn samples from non contaminated (blank) samples (Kos et al. 2003, 2004, 2007; Delwiche and Hareland 2004).

Papers on the application of FT-IR spectroscopy for a determination of DON content in grain mostly exploit the range below 4000 cm^{-1}. In particular, Mossoba et al. (1996) reported DON characteristic absorption bands between 1244 and 1750 cm^{-1}. Some additional bands at ca. 3500 and close to 3650 cm^{-1} (Young and Games 1994) and not far from 1700 cm^{-1} (Abramovic et al. 2007) have been reported. The most recent theoretical calculations of IR bands cover the MIR range below 4000 cm^{-1} (Turker and Gumus 2008), also used by Kos et al. (2007) for corn analysis. Therefore the identification of any spectral features of DON within the NIR range is highly desirable. FT-IR spectroscopy allows to perform rapid measurements with an excellent signal-to-noise ratio and high resolution.

The aim of the present work was to investigate the feasibility of using FT-NIR for the rapid and non-invasive determination of DON in durum and common wheat.

Materials and methods

Sample preparation. A total of 262 wheat (143 durum and 119 common) samples, belonging to 32 different varieties, were obtained from wheat naturally infected by *Fusarium graminearum* and *Fusarium culmorum* in experimental fields in Northern Italy

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during the period from 2002 to 2006. Wheat samples were ground with a Tecator Cyclotec 1093 (International PBI, Hoganas, Sweden) laboratory mill equipped with a 500 µm sieve, and were analyzed by HPLC and by FT-NIR spectroscopy. DON levels, as established by HPLC analysis, ranged from 50 to 2600 µg kg^{-1} in durum wheat and from 50 to 3000 µg kg^{-1} in common wheat. Samples containing less than 50 µg kg^{-1} DON (the quantification limit of the HPLC-method) were considered as DON-free. Mean recoveries of DON from durum and common wheat samples (n = 3) spiked at levels of 200, 800 and 1500 µg kg^{-1} were 75-93% with RSD_r of 1.4-14.2% for durum wheat and 79-83% with RSD_r of 1.1-12.2% for common wheat. To investigate the distribution of DON during sieving, five durum wheat samples were ground by a Bühler MLI 204 (Bühler S.p.A., Milan, Italy) to pass a 1-mm sieve and analyzed by HPLC for DON. A fraction of these samples was sieved to pass a 300 µm sieve, and the two fractions (below and above 300 µm) were analyzed by HPLC.

HPLC quantitative analysis. HPLC quantitative analysis was performed to obtain the reference value of the DON content for all wheat samples tested herein. Wheat samples were analyzed according to the procedure described by Visconti et al. (2004) with minor modifications. Briefly, 25 g of ground wheat was extracted with 100 mL of phosphate buffer solution (PBS, 10 mM sodium phosphate, 0.85% sodium chloride, pH 7.4) by blending at high speed for 2 min with a Sorvall Omnimixer (Sorvall Instruments, Norwalk, Conn.). The extract was filtered through both filter paper (Whatman no. 4) and glass microfiber filter (Whatman GF/A) and 2 mL of the filtered extract were cleaned up by DONTest™ (Vicam, Watertown, MA, USA). After washing the column by passing 5

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mL water through it, DON was eluted with 1.5 mL methanol. The eluate was dried under nitrogen stream at 50°C, re-dissolved in 200 µL of mobile phase (acetonitrile:water, 10:90, v/v) and 50 µL were injected onto an LC system with ultraviolet diode array detector set to 220 nm.

FT-NIR analysis. FT-NIR spectra were recorded using an Antaris II FT-NIR spectrophotometer (Thermo Electron Corporation, Madison, WI, USA) equipped with an interferometer (containing a fixed and a moving mirror, a beam-splitter), an integrating sphere working in diffuse reflection, and an indium and gallium arsenide (InGaAs) detector. A sample cup spinner allowed the automatic collection of several subsamples from each sample. These subsamples were averaged to obtain representative spectra of relatively heterogeneous samples. The integrating sphere's internal reference was also used to collect the background spectrum. The moving mirror speed was set at 1.63 scans/sec. Approximately 30 g of ground wheat samples were placed into the rotating sample cup spinner with a quartz cup. Spectroscopic data were recorded as absorbance between 10000-4000 cm^{-1} with 8 cm^{-1} resolution to give a total of 750 points per sample. Each sample spectrum, consisting of an average of 128 consecutive interferometer scans, was collected in about 1 min 20 s and stored in a separate data file.

FT-NIR transmission spectra of DON were obtained using the Transmission module (Thermo Electron Corporation) consisting of a sample holder to allocate glass tubes. Approximately 1000 µl of solution were transferred into glass culture tubes (6 x 50 mm). Spectroscopic data were recorded as absorbance between 10000-4000 cm^{-1} with 8 cm^{-1}

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resolution and each sample spectrum consisted of an average of 16 consecutive interferometer scans.

Commercially available crystalline DON standard (Sigma, Milan, Italy) was dissolved in acetonitrile to prepare a 1 mg ml^{-1} standard solution. FT-NIR transmission spectrum of DON was determined after subtraction of the mean spectrum (n = 10) of acetonitrile from the mean spectrum (n = 10) of DON standard solution. Furthermore, two sub-samples of a DON-free durum wheat sample (< 50 µg kg^{-1}) were spiked with acetonitrile and with the same volume of DON standard solution (spiking level 10000 µg kg^{-1}), respectively. The FT-NIR spectrum was obtained after subtraction of the spectrum of acetonitrile spiked wheat from that of DON spiked wheat. Similarly, another FT-NIR spectrum was obtained after subtraction of a DON-free durum wheat spectrum from that of a naturally contaminated one (13000 µg kg^{-1} DON).

Multivariate data analysis. Based on DON content (as determined by HPLC analysis) wheat samples were distributed into classes each covering 100 µg kg^{-1} DON. Calibration and validation samples were randomly selected from each class by considering an approximate ratio of 2.5:1 between calibration and validation samples. Spectroscopic data were processed by TQ Analyst® professional edition (Thermo Electron Corporation). The Spectrum Outlier diagnostic (TQ Analyst®) was run to seek sample spectra that were the most different from the other samples. In particular, it was calculated the mean spectrum for all calibration samples, measured the distance between the mean spectrum and the spectrum of each calibration sample. The Chauvenet Test estimated whether the

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difference between spectra was significant. Once the initial population was free of spectral outliers calibration models were developed and statistically elaborated for quantitative analysis using PLS regression with full cross validation (*leave one out*). DON data obtained by FT-NIR were transformed using Standard Normal Variate (SNV) (Barnes et al., 1989), first derivative, mean centering and Savitzky-Golay smoothing (2nd-order polynomial filtering operation performed on 3 points) (Savitzky and Golay, 1964), and correlated with those obtained by HPLC.

The Partial Least Squares (PLS) models performance were evaluated by calculating the correlation coefficient (*r*) between the reference (HPLC data) and the predicted (FT-NIR data) DON levels, the root mean square error of calibration (RMSEC), the root mean square error of cross-validation (RMSECV) and the number of PLS factors. External data sets (validation sets) were also used to evaluate the performance of the models for prediction, and the root mean square error of prediction (RMSEP) values were calculated. Validation sets were selected to cover the range of DON-content of interest. RMSEC, RMSECV and RMSEP were expressed by the same DON level units ($\mu g\ kg^{-1}$) in wheat samples. In the development of all calibration models, 10 PLS factors were set up as a maximum number to work with. The optimum number of PLS factors was automatically calculated by the TQ Analyst® software corresponding to the lowest number of factors giving the closest to minimum value of the prediction residual error sum of squares (PRESS). As the calculated number of PLS factors gave models that strictly dependent on calibration samples, an appropriate number was chosen giving comparable RMSEC, RMSECV and RMSEP values.

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The accuracy of each model was evaluated based on the coefficient of determination (r^2) for predicted *versus* reference DON levels in calibration and validation sets. It was assumed that with r^2 = 0.50-0.65 discrimination between high and low values was possible, r^2 = 0.66-0.81 allowed approximate quantitative prediction, r^2 = 0.82-0.90 allowed good prediction, and excellent calibration model were obtained with r^2 = 0.91 or higher (Williams 2003). The prediction accuracy of models was also evaluated based on the residual predictive deviation (RPD), defined as the ratio of the standard deviation of the reference DON values in the validation set to the RMSEP. In general, RPD values of 3.1-4.9 indicate NIR calibration models suitable for screening purposes, whereas values of 5.0-6.4 are considered adequate for quality control (Fearn 2002). However, a more qualitative interpretation of the RPD considers values lower than 1.5 insufficient for most applications and values greater than 2 as excellent (Williams 2001; Smyth et al. 2008).

The range error ratio (RER), calculated by dividing the range of the reference DON values in the validation set by the RMSEP, was used as a useful indicator to assess the practical utility of the calibration as a predictive model. Models with RER of less than 3 had a little practical utility; between 3 and 10 had limited to good practical utility; and above 10 indicated a high practical utility value (Fearn 2002; Williams 2003).

Qualitative analysis of different wheat cultivars. The ability of FT-NIR spectroscopy to discriminate between blank and naturally contaminated samples of different cultivars of both durum and common wheat was evaluated by Discriminant Analysis. Based on the DON content as determined by HPLC analysis, samples were split into two classes, one including samples with less than 300 µg kg^{-1} DON (assumed as "blank") and the other

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one including naturally contaminated (from 300 µg kg^{-1} up to about 3000 µg kg^{-1} DON) wheat samples. Validation samples were randomly selected to cover the range of interest (up to 2000 µg kg^{-1}). Calibration and validation sets contained samples in the approximate ratio of 3:1. All spectroscopic data were transformed using variance scaling normalization, Standard Normal Variate (SNV) (Barnes et al., 1989), first derivative, mean centering and Savitzky-Golay smoothing (2nd-order polynomial filtering operation performed on 5 points) (Savitzky and Golay, 1964).

Discriminant Analysis was performed to create the distribution model by using the spectral information from all calibration samples. In particular, during calibration, the software computed an average spectrum and then generated a distribution model by estimating the variance at each frequency in the analysis range. When the calibrated method was used to analyze validation samples, the software performed a Principal Component Analysis (PCA) on the validation sample spectra and on the variance spectra to determine score values. The scores were used to produce Mahalanobis distance values, which in turn were used to rank the classes. Discriminant Analysis assigned the ranking class based on the level of similarity with the spectrum of the validation sample. The Mahalanobis distance between the validation sample and each class center was also recorded.

Results and discussion

Effect of sample preparation on DON analysis by FT-NIR analysis. Kos et al. (2003; 2007) reported that the repeatability of spectral measurements performed on ground

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maize (particle size < 1 mm) was not good enough for attempting classification by MIR-ATR of DON contaminated maize, and that a sieving procedure (selecting particles with diameter between 250 and 100 µm) improved the repeatability of spectral measurements from 20% to 4.4%.

As an initial approach, ground wheat samples were sieved to pass a 300 µm sieve and analysed by FT-NIR spectroscopy. An alternative sample preparation was used to avoid the time-consuming sieving step (20-30 min if carried out after grinding). In particular, wheat samples were milled with a Tecator Cyclotec laboratory mill (Tecator Cyclotec) equipped with a 500 µm sieve and the obtained fraction was directly analysed by FT-NIR spectroscopy. Spectra for 50 repetitive measurements of a sieved sample were compared to those of an unsieved sample of the same wheat variety and with a comparable DON-content.

In order to statistically evaluate spectra differences between wheat samples with particle size < 300 µm and < 500 µm, the analysis of variance (ANOVA, one way) was applied to the two groups of repetitive measurements. No significant difference was found within each group, while a significant difference ($F = 29.62$, $F_{critic} = 3.84$) was observed between the mean spectra of the two groups due to the different distribution of particle sizes and relevant chemical composition. The PCA score/score plot revealed that the cluster containing samples with particle size < 300 µm was more scattered than the cluster containing samples with particle size < 500 µm, showing a more homogeneous distribution of particle size in the unsieved sample (Figure 1).

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[Insert Figure 1 about here]

Furthermore, the HPLC analysis of different fractions obtained after sieving at 300 µm of five different wheat samples showed a clear segregation of DON between fractions with respect to the original ground wheat (Table I). In particular, mean DON levels in the samples dropped by 84% in wheat fractions with particle size > 300 µm, and accumulated by 115% in fractions with particle size < 300 µm.

[Insert Table I about here]

Consequently, the sample preparation approach performed by using a Tecator Cyclotec milling at < 500 µm was preferred in order to avoid both the time-consuming sieving step, and the erroneous DON determination due to the different distribution in the sieved fractions.

DON absorption in the NIR region. The major FT-NIR absorption bands of DON dissolved in acetonitrile (Figure 2, line a) were identified in the range of 7400-7100 cm^{-1} with a peak at 7095 cm^{-1}, and between 5000 and 5500 cm^{-1} with a peak at 5251 cm^{-1}. The absorption band at 5251 cm^{-1} was much stronger than that at 7095 cm^{-1}. The spectrum obtained after subtraction of the spectrum of acetonitrile spiked wheat from that of DON spiked wheat showed absorption bands at about 7000 and 5250 cm^{-1} (Figure 2, line b) corresponding to those of DON spectrum in acetonitrile. The increased intensity of the

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band at 7000 cm^{-1} was due to an overlapping of DON and water absorption bands. The same absorption bands were observed in the FT-NIR spectrum obtained after subtraction of a DON-free wheat spectrum from that of a naturally contaminated one (Figure 2, line c).

[Insert Figure 2 about here]

DON levels in wheat are generally several orders of magnitude lower than levels of the main wheat constituents (protein, starch, etc.), and spectra recorded for contaminated and uncontaminated wheat may differ one from another more because of difference in chemical composition (due to development of the fungus) than because of DON content. Thus, subtraction of the spectra can produce spurious features not necessarily related to DON content due to the relatively low DON levels commonly occurring in wheat. However, the use of highly contaminated wheat (at levels higher than 10000 µg kg^{-1}) produced subtractive spectra with appreciable FT-NIR spectral bands (particularly at 5251 cm^{-1}) related to DON as shown in Figure 3. The characteristic absorption bands of DON could not be identified in wheat samples contaminated at lower levels, such as those used herein (up to 3000 µg kg^{-1}) for the three PLS regression models. Nevertheless, a certain discrimination between low and high DON levels was obtained by considering the entire spectral range between 10000 and 4000 cm^{-1} (see below).

DON analysis by FT-NIR spectroscopy. Three PLS regression models were built by considering the spectral range between 10000 and 4000 cm^{-1} for durum, common and

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durum+common wheat samples, respectively. Table II summarizes sample statistics for the calibration and validation set of durum, common and durum+common wheat. Comparing the mean DON levels (ranging from 439 to 887 µg kg^{-1}) with median levels (from 188 to 656 µg kg^{-1}) for durum or common wheat samples, a very skewed data distribution was observed, with many small values and fewer large ones. After combination of the two wheat species (durum+common wheat) a better distribution of DON level was obtained and the resulting mean (585-750 µg kg^{-1}) and median (395-523 µg kg^{-1}) DON levels were closer.

[Insert Table II about here]

Validation sets of common and durum wheat were selected, both consisting of 30 samples. The two PLS models were validated up to about 2000 µg kg^{-1} DON to cover the range of regulatory levels set by the EU for unprocessed wheat and most wheat based products. The selection was performed based on DON content (measured by HPLC) in order to have as many samples as possible of the validation set within the range of DON content to be considered for the calibration (Table II).

Running the PLS analysis on the calibration sets a positive linear correlation ($r > 0.90$) was obtained in the measured range for both models, indicating a god fit between HPLC and FT-NIR data. According to the coefficients of determination (r^2) and slope values, both > 0.82, a good prediction of DON content by FT-NIR spectroscopy was achieved in both PLS calibration models (Figure 3a and 3b).

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[Insert Figure 3 about here]

Furthermore, RMSECV values were 470 and 516 µg kg^{-1} respectively, both corresponding to 4 PLS factors (Table III). The performance of these models for DON prediction on the validation sets gave RMSEP values of 306 µg kg^{-1} for durum wheat and 348 µg kg^{-1} for common wheat. These values were not too far from RMSECV values obtained in cross-validation. The r^2 values of the validation sets (0.62-0.63) indicated that the PLS models were able to discriminate between low and high DON levels in durum and common wheat. An improvement of RMSECV and r^2 values was obtained by using the recommended number of PLS factors (5-6), however the obtained PLS models quantified the calibration samples with great accuracy but did not produce the same level of accuracy when applied to validation samples and gave RMSEP and RMSECV values that were too far from RMSEC. Based on these findings, it was decided to run the calibrations with a low number of factors in order to obtain comparable values of RMSEC, RMSEP and RMSECV.

[Insert Table III about here]

In addition to the RMSECV, r^2 and RMSEP, the PLS models were evaluated using the RER and RPD values. Both the RER and RPD standardize the RMSEP value of the model against the range and the standard deviation of the reference data in the validation set. RPD values obtained for durum and common wheat (1.6-2.0) show the ability of the

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model to discriminate between high and low contaminated samples. However, it is evident that a further improvement of RPD values, and then more accurate PLS models, could be obtained by increasing the range and the distribution of DON content in the validation samples. On the other hand, RER values lie within 6 and 7, indicating a good practical utility of the models for DON prediction in wheat samples (Table III).

Results obtained in the present study were similar to those reported by Pettersson and Aberg (2003) in terms of correlation coefficient, slope and RMSEP. However, a remarkable improvement was obtained with the PLS regression models used herein for prediction of DON in durum and common wheat because calibrations were made on a larger number of calibration and validation samples. Furthermore DON contamination levels used herein were close to the DON maximum limits set by the European Union for unprocessed durum (1750 µg kg^{-1}) and common (1250 µg kg^{-1}) wheat, whereas Pettersson and Aberg (2003) calibrated the models at higher and less realistic levels (up to 10000-15000 µg kg^{-1}).

An additional PLS calibration model was built by combining durum and common wheat samples to evaluate the possibility to include different wheat species belonging to different varieties in the same model. Also in this case a good fit of the PLS model (r = 0.84) was obtained (Figure 3, Table III). The r^2 values indicated that the model approximated quantitative predictions of DON in the calibration set (r^2 = 0.71) and allowed for discrimination between high and low DON levels in the validation set (r^2 = 0.58) (Table III). These results were also confirmed by the RER value (6.3), while the RPD value (1.0) failed to attain the minimum recommended for quantitative analysis.

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Although results obtained in the present study are not fully satisfying for quantitative or semi-quantitative analysis, they provide a good evidence of the feasibility of using FT-NIR spectroscopy as a screening tool for determination of DON in wheat samples. Furthermore, considering that different wheat species (durum and common) and wheat varieties contain different amount of chemical constituents, such as protein, lipids and moisture with remarkable spectral differences (Cocchi et al., 2006; Morris et al., 2005), our findings indicate that this variability does not affect the applicability of FT-NIR for DON analysis

FT-NIR qualitative analysis of different wheat cultivars. The ability of FT-NIR spectroscopy to discriminate between blank and naturally contaminated durum and common wheat samples of different cultivars was evaluated by performing a discriminant analysis on 197 calibration samples. In particular, depending on DON content calculated by HPLC analysis, samples were split into two classes, one including 84 "blank" samples (up to 300 µg kg^{-1} DON) and the other one including 113 "contaminated" samples (> 300 µg kg^{-1} DON). The samples were classified as members of each group based on the lowest corresponding Mahalanobis distance, i.e. the multi-dimensional space whose boundaries determine the range of variation.

[Insert Figure 4 about here]

Figure 4 shows graphically the Mahalanobis distance between each sample and the "blank" and "contaminated" classes that were selected for the X- and Y- axis of the plot,

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respectively. The boundaries, displayed as perpendicular lines in the plot, show the 95% confidence intervals for the two classes. All calibration and validation samples relevant to the "blank" class are expected to be clustered in the upper left corner of the plot, while samples falling in the lower right corner define the "contaminated" class. As observed in Figure 4, a few calibration samples of the two classes fall in the uncertainty area (lower left corner, 40 out of 197). Most of them were "blank" (16%) or contained DON levels between 300 and 500 µg kg^{-1} (30%), indicating that the model had a limited degree of specificity for wheat samples contaminated with DON levels close to the cut-off limit. When the obtained model was applied to a set of 65 validation samples, it correctly classified 69% of samples as "blank" or "contaminated" wheat. The remaining 20 samples were incorrectly classified as false positive or negative sample. Also in this case, most of misclassified samples were "blank" (55%) or contained DON levels ranging from 300 to 400 µg kg^{-1} (25%), confirming that the goodness of the discrimination model was greatly influenced by the low DON cut-off limit fixed to distinguish the two classes. Lower cut-off levels, i.e. 50 µg kg^{-1} (the quantification limit of the HPLC method), 100 and 200 µg kg^{-1}, led to lesser precise models, while higher cut-off levels gave poorly reliable models due to problems associated with the relatively limited number of samples with high DON levels. However, these results still recommend the use of FT-NIR as a sorting tool for screening purpose, since the legal limit at present is well over 300 µg kg^{-1} for most commodities (with the important exception of baby food where the limit is 200 µg kg^{-1}). An improvement to this qualitative model could be obtained by increasing the number of samples, and in particular, by increasing the number of high DON contaminated samples.

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Conclusions

Performances of Discriminant Analysis for qualitative models and of PLS regression models for semi-quantitative prediction for DON contamination of wheat makes FT-NIR analysis a useful tool to discriminate between high and low DON contaminated samples. FT-NIR analysis is suitable for the determination of DON in a specific wheat species (durum or common) or in both at levels far below the DON maximum permitted limits set by the European Commission for unprocessed wheat. FT-NIR analysis might be used for rapid, non invasive, inexpensive and user-friendly screening of large numbers of wheat samples.

The FT-NIR models developed herein merit further implementation in a larger study involving more calibration and validation samples with a uniform distribution of DON levels

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References

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Abramovic B, Jajic I, Abramovic B, Cosic J, Juric V. 2007. Detection of deoxynivalenol in wheat by Fourier Transform Infrared Spectroscopy. Acta Chimica Slovenica 54:859-867.

Barnes BJ, Dhanoa MS, Lister SJ. 1989. Standard Normal Variate Transformation and De-trending of Near-Infrared Diffuse Reflectance Spectra. Applied Spectroscopy 43:772-777.

Canady RA, Coker RD, Egan SK, Krska R, Kuiper-Goodman T, Olsen M, Pestka J, Resnik S, Schlatter J. 2001. Deoxynivalenol. In: Safety evaluation of certain mycotoxins in food. Fifty-sixth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), FAO Food and Nutrition paper 74, WHO Food Additives ser. 47. World Health Organization, Geneva. p 419.

Cocchi M, Durante C, Foca G, Marchetti A, Tassi L, Ulrici U. 2006. Durum wheat adulteration detection by NIR spectroscopy multivariate calibration. Talanta 68:1505-1511.

Delwiche SR, Hareland GA. 2004. Detection of scab-damaged hard red spring wheat kernels by near-infrared reflectance. Cereal Chemistry 81:643-649.

Dowell FE, Ram MS, Seitz MS. 1999. Predicting scab, vomitoxin, and ergosterol in single wheat kernels using near-infrared spectroscopy. Cereal Chemistry 76:573-576.

European Commission (2007). Commission regulation (EC) No 1126/2007 of 28 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. Official Journal of European Union. L 255:14-17.

Fearn T. 2002. Assessing calibrations: SEP, RPD, RER and R^2. NIR news 13:12-14.

For Peer Review Only

Kos G, Lohninger H, Krska R. 2003. Development of a method for the determination of *Fusarium* fungi on corn using mid-infrared spectroscopy with attenuated total reflection and chemometrics. Analytical Chemistry 75:1211-1217.

Kos G, Krska R, Lohninger H, Griffiths PR. 2004. A comparative study of mid-infrared diffuse reflection (DR) and attenuated total reflection (ATR) spectroscopy for the detection of fungal infection on RWA2-corn. Analytical and Bioanalytical Chemistry 378:159-166.

Kos G, Lohninger H, Mizaikoff B, Krska R. 2007.Optimisation of a sample preparation procedure for the screening of fungal infection and assessment of deoxynivalenol content in maize using mid-infrared attenuated total reflection spectroscopy. Food Additives and Contaminants 24 (7):721-729.

Lippolis V, Pascale M, Visconti A. 2006. Optimization of a fluorescence polarization immunoassay for rapid quantification of deoxynivalenol in durum wheat-based products. Journal of Food Protection 69:2712-2719.

Morris CF, Campbell KG, King GE. 2005. Kernel texture differences among US soft wheat cultivars. Journal of the Science of Food and Agriculture 85 (11):1959-1965.

Mossoba MM, Adams S, Roach JAG, Trucksess MW. 1996. Analysis of trichothecene mycotoxins in contaminated grains by gas chromatography/matrix isolation/Fourier transform infrared spectroscopy and gas chromatography/mass spectrometry. Journal of AOAC International 75 (5):1116-1123.

Mouazen AM, De Baerdemaeker J, Ramon H. 2005. Towards development of on-line soil moisture content sensor using a fibretype NIR spectrophotometer. Soil Tillage Research 80:171–183.

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Pettersson H, Aberg L. 2003. Near infrared spectroscopy for determination of mycotoxins in cereals. Food Control 14: 229-232.

Savitzky A, Golay MJE. 1964. Smoothing and differentiation of data by simplified least squares procedures. Analytical Chemistry 36:1627-1639.

SCF (Scientific Committee on Food) 2002. Opinion of the Scientific Committee on Food on Fusarium toxins. Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol, adopted on 26 February 2002. European Commission. Available from: http://europa.eu.int/comm/food/fs/sc/scf/out123_en.pdf.

Schlatter J. 2004. Toxicity data relevant for hazard characterization. Toxicology Letters 153:83-89.

Schothorst R, van Egmond H. 2004. Report from Scoop task 3.2.10. Collection of occurrence data of *Fusarium* toxins in food and assessment of dietary intake by the population of EU members states. Subtask: trichothecenes. Toxicology Letters 153:133-143.

Smyth HE, Cozzolino D, Cynkar WU, Dambergs RG, Sefton M, Gishen M. 2008. Near infrared spectroscopy as a rapid tool to measure volatile aroma compounds in Riesling wine: possibilities and limits. Analytical and Bioanalytical Chemistry 390:1911-1916.

Turker L, Gumus S. 2008. A theoretical study on vomitoxin and its tautomers. Journal of Hazardous Material, Article in Press. DOI: 10.1016/j.jhazmat.2008.06.087

Young GC, Games DE. 1994. Analysis of Fusarium mycotoxins by gas chromatography-Fourier transform infrared spectroscopy. Journal of Chromatography, 663(2): 211-218.

For Peer Review Only

Williams, PC. (2001). Implementation of near-infrared technology. In Near-infrared technology in the agricultural and food industries. 2nd edition, P. Williams & K. Norris (Eds.), St. Paul, MN: American Association of Cereal Chemists. p. 145.

Williams, P. 2003. Near-infrared technology-Getting the best out of light. In: A short course in the practical implementation of near infrared spectroscopy for the user. 1.1 PDK Projects Inc., editors. Nanaimo, Canada. p 109.

Visconti A, Haidukowski EM, Pascale M, Silvestri M. 2004. Reduction of deoxynivalenol during durum wheat processing and spaghetti cooking. Toxicology Letters 153

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Figure 1. Score/score plot of spectral data (PC1 vs PC2) from 50 repetitive measurements of two wheat samples with different particle sizes. The two clusters represent wheat samples with particle size < 500 µm (left, unsieved) and < 300 µm (right, sieved).

Figure 2. FT-NIR subtractive spectra of deoxynivalenol in acetonitrile spectrum from that of acetonitrile (line a), of acetonitrile spiked wheat spectrum from that of DON spiked (10000 µg kg^{-1}) wheat (line b) and of deoxynivalenol-free wheat spectrum from that of naturally contaminated (13000 µg kg^{-1}) wheat.

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Figure 3. PLS regression models of deoxynivalenol content determined by FT-NIR and the reference method (HPLC) of durum (3 a), common (3 b) and durum+common (3 c) wheat samples.

Figure 4. Pairwise distance plot reporting the Mahalanobis distance between each sample and the "Blank" and "Contaminated" classes.

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Figure 1.

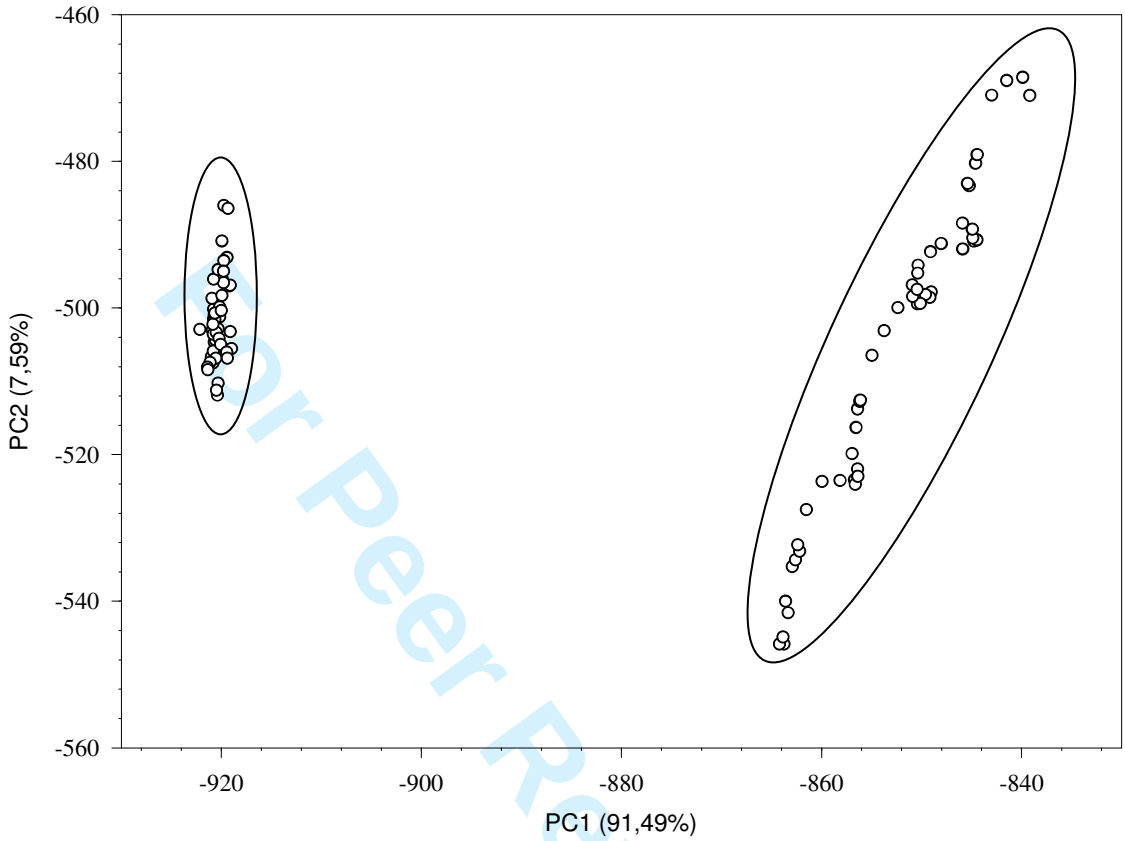

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Figure 2.

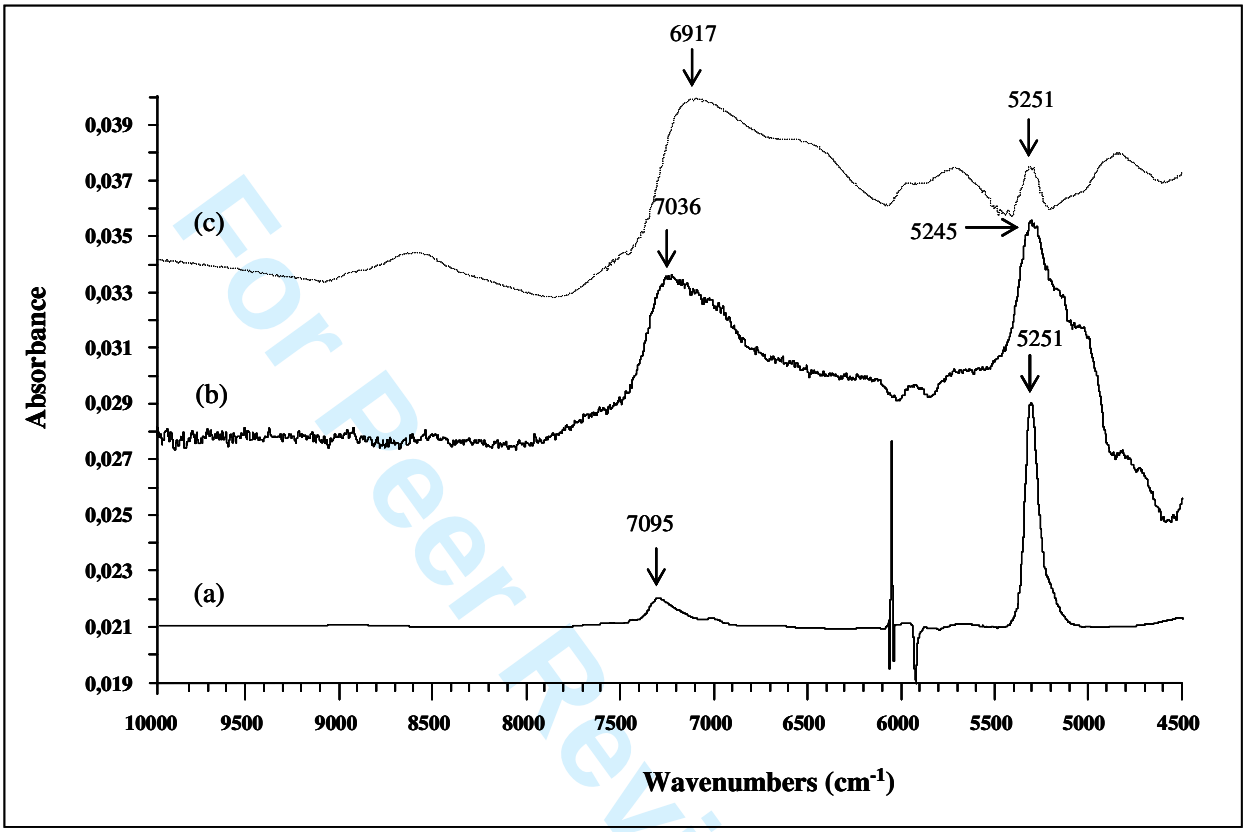

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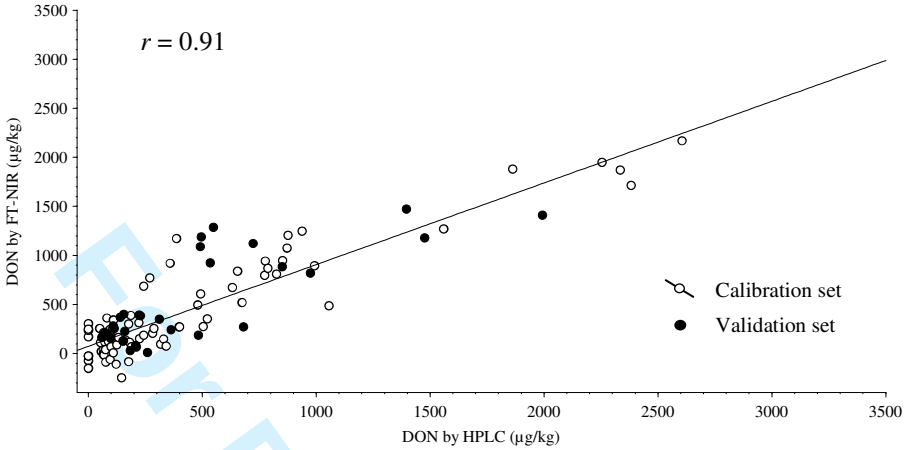
Figure 3 (a)



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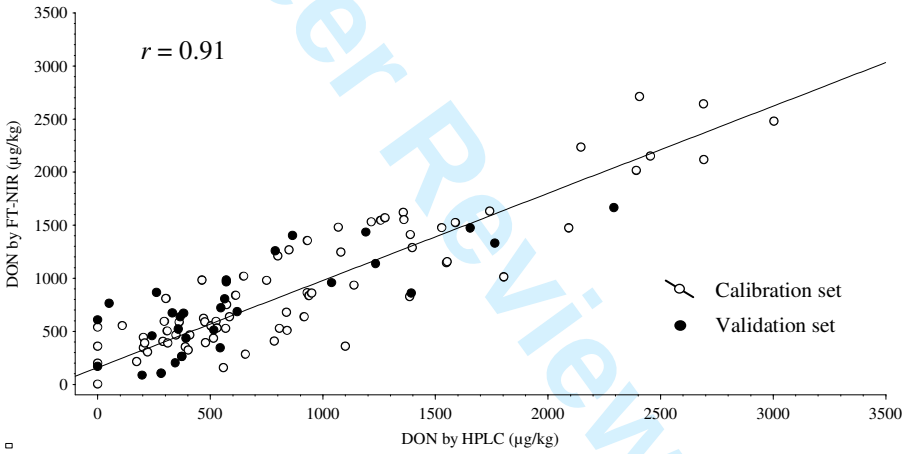

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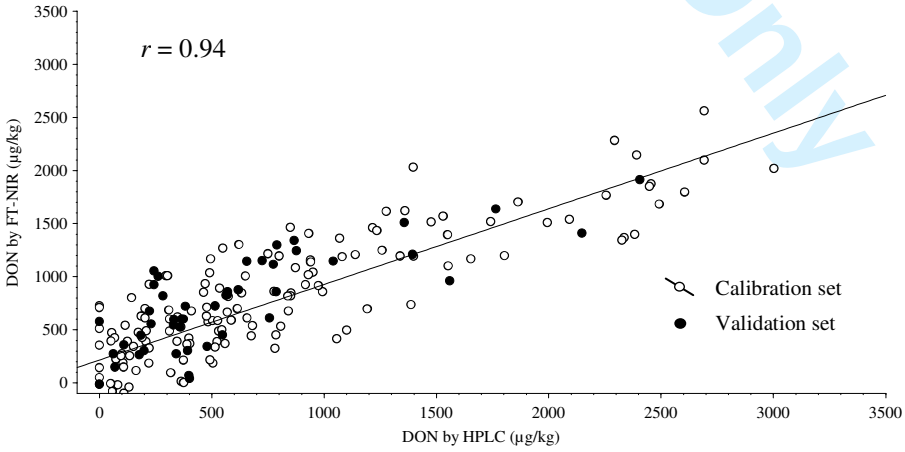

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Figure 4.

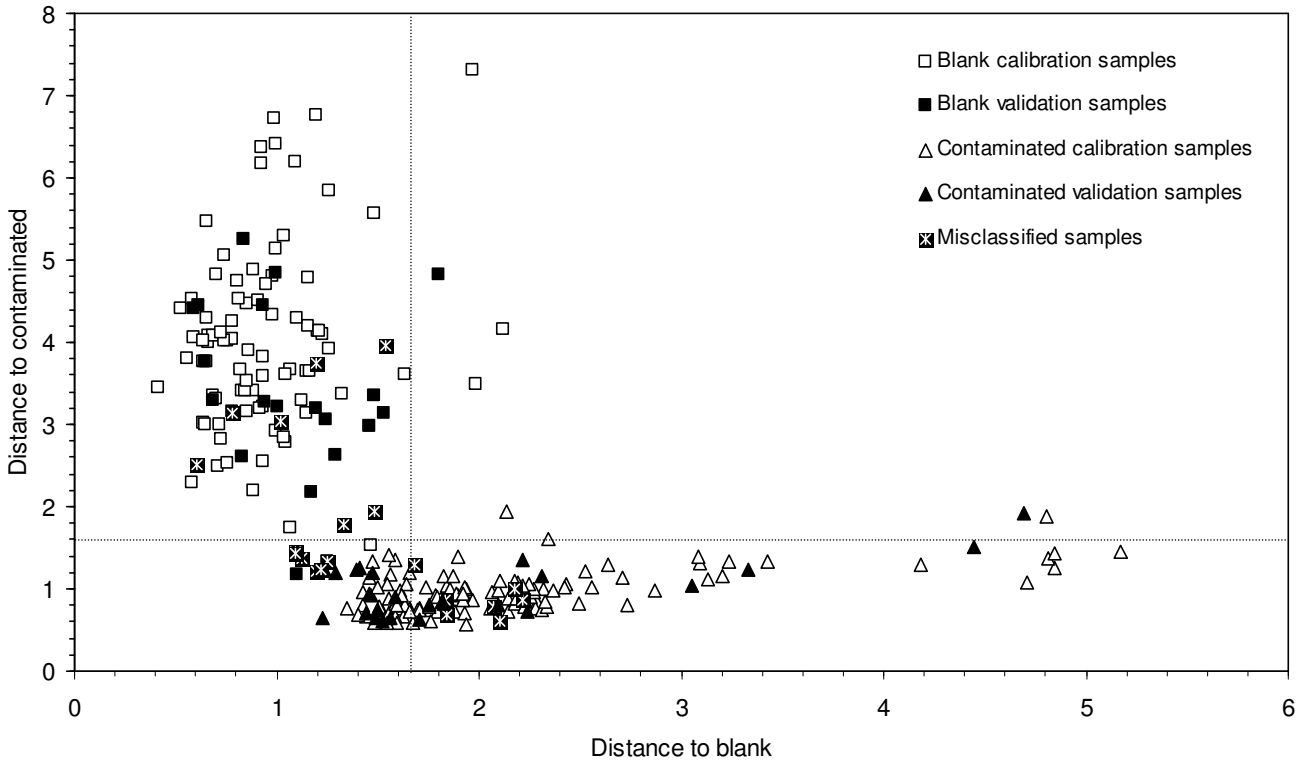

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Table I. Deoxynivalenol content in ground durum wheat prior and after sieving (300 µm sieve).

Wheat sample (#)	Unsieved (µg/kg)	Sieved, < 300 µm (µg/kg)	Recovery (%)*	Sieved, > 300 µm (µg/kg)	Recovery (%)*
#1	813	995	122	577	71
#2	1466	1619	110	1226	84
#3	819	886	108	696	85
#4	776	846	109	751	97
#5	1277	1588	124	1085	85
Mean ± SD			115 ± 8		84 ± 9

*Percentage of deoxynivalenol in the sieved fraction relative to the original (unsieved) wheat sample.

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Table II. Statistical summary of deoxynivalenol content by HPLC in the calibration and validation sets of durum, common and durum+common wheat with particle size < 500 µm.

	Durum	Common	Durum+common	Durum	Common	Durum+common
	Calibration set			Validation set		
N. of samples	76	77	149	30	30	48
Range (µg/kg)	0-2600	0-3000	0-3000	60-1990	0-2290	0-2400
Median (µg/kg)	188	656	523	245	530	395
Mean (µg/kg)	439	887	750	459	658	585
SD* (µg/kg)	590	720	722	470	681	533

*SD = standard deviation

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Table III. Calibration and validation results for deoxynivalenol detection by FT-NIR in durum, common and durum+common wheat with particle size < 500 µm.

Parameters*	Durum	Common	Durum+common
$Slope_{calibration}$	0.83	0.82	0.71
$r_{calibration}$	0.91	0.91	0.84
$r^2_{calibration}$	0.83	0.82	0.71
$Slope_{validation}$	0.79	0.62	0.71
$r_{validation}$	0.80	0.79	0.76
$r^2_{validation}$	0.62	0.63	0.58
RMSEP (µg/kg)	306	348	379
RMSECV (µg/kg)	470	516	555
PLS factors	4	4	5
RER	6.3	6.6	6.3
RPD	1.6	2.0	1.0

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* r^2 determination coefficient; RMSECV, root mean square error of cross-validation; RMSEP, root mean square error of prediction set; PLS, partial least squares; RER, ratio error range; RPD, residual predictive deviation.

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RMSEC (μg/kg)	240	303	386

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