



HAL
open science

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

Annalisa de Girolamo, Vincenzo Lippolis, Erik Nordkvist, Angelo Visconti

► **To cite this version:**

Annalisa de Girolamo, Vincenzo Lippolis, Erik Nordkvist, Angelo Visconti. Rapid and non invasive analysis of deoxynivalenol in durum and common wheat by Fourier-Transform Infrared (FT-NIR) spectroscopy. *Food Additives and Contaminants*, 2009, 26 (06), pp.907-917. 10.1080/02652030902788946 . hal-00573870

HAL Id: hal-00573870

<https://hal.science/hal-00573870v1>

Submitted on 5 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



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

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2008-304.R1
Manuscript Type:	Original Research Paper
Methods/Techniques:	Analysis - NIR, Screening assays, Statistical analysis
Additives/Contaminants:	Mycotoxins - trichothecenes
Food Types:	Cereals and grain

SCHOLARONE™
Manuscripts

1
2
3 **Rapid and non-invasive analysis of deoxynivalenol in durum and**
4 **common wheat by Fourier-Transform Infrared (FT-NIR) spectroscopy**
5
6
7
8
9

10
11 A. De Girolamo¹, V. Lippolis¹, E. Nordkvist², & A. Visconti¹
12

13
14
15
16 ¹Institute of Sciences of Food Production (ISPA), National Research Council (CNR), via
17
18 G. Amendola 122/O, 70126 Bari, Italy,
19

20
21 ²National Veterinary Institute, SE-751 89 Uppsala, Sweden.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39

40 **Running title:** FT-NIR analysis of deoxynivalenol in wheat
41
42
43

44 Correspondence: A. De Girolamo. e-mail: annalisa.degirolamo@ispa.cnr.it
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 $\mu\text{g kg}^{-1}$) were comparable and not too far from values of root mean square error of cross-validation (RMSECV, 470-555 $\mu\text{g kg}^{-1}$). 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 $\mu\text{g kg}^{-1}$ 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.

1
2
3 **Keywords:** FT-NIR spectroscopy; deoxynivalenol; wheat; PLS regression, discriminant
4 analysis.
5
6
7
8
9

10 **Introduction**

11
12
13
14 Deoxynivalenol (DON) is a type B trichothecene mycotoxin produced by fungi of the
15 *Fusarium* genus, in particular *Fusarium graminearum* (Gibberella zea) and *Fusarium*
16 *culmorum*. It is frequently associated with contaminated cereal crops such as wheat,
17 maize, barley, oats, rye and less often with rice, sorghum and triticale (Canady et al.
18 2001; Schothorst and van Egmond 2004). DON inhibits the synthesis of DNA, RNA and
19 proteins, and has a hemolytic effect on erythrocytes. In animals, acute/subacute oral
20 toxicity of DON is characterized by vomiting, feed refusal, weight loss and diarrhea
21 (Schlatter 2004).
22
23
24
25
26
27
28
29
30
31
32
33
34
35

36 Based on the temporary tolerable daily intake (TDI) of 1 µg/kg body weight established
37 by the EU Scientific Committee on Food (SCF) (SCF 2002), the European Commission
38 has set maximum permitted levels for DON in a variety of cereal based foods (European
39 Commission 2007). In particular, 1750 µg kg⁻¹ and 1250 µg kg⁻¹ were set as maximum
40 levels for unprocessed durum and common wheat, respectively; lower levels were set for
41 wheat flour, bran, germ and pasta (750 µg kg⁻¹), for wheat and wheat products for direct
42 human consumption (750 µg kg⁻¹ and 500 µg kg⁻¹), and for wheat-based infants and baby
43 food (200 µg kg⁻¹).
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Rapid and sensitive methods for the quantification of DON in wheat and wheat-based
4 food products are strongly demanded by private and public quality control laboratories,
5 as well as in surveys aiming to provide data for risk assessments. Several methods based
6 on competitive enzyme-linked immunosorbent assays (ELISA) have been developed for
7 the rapid screening and quantification of DON in cereals. ELISA methods are sensitive,
8 rapid and easy-to-use, but they often show strong cross-reactivity against DON acetyl
9 derivatives, require long incubation times for complete antigen-antibody reaction and
10 commercial kits are still rather expensive. A fluorescence polarization (FP) immunoassay
11 has been recently developed for the rapid and quantitative analysis of DON in durum
12 wheat, semolina, and pasta at levels below the EU maximum permitted levels (Lippolis et
13 al. 2006).

14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32 Although these methods can be used for screening purposes, they are destructive and
33 require preparation and extraction steps. In the past few decades, many researchers have
34 focused on the potential use of infrared spectroscopy in the analysis of food and feed,
35 both in the mid- and near-infrared regions. Infrared spectroscopy (IR) is a rapid analytical
36 technique requiring very little labour, once a calibration is obtained, and no sample
37 extraction and, therefore, does not create chemical waste.

38
39
40
41
42
43
44
45
46
47
48 Petterson and Aberg (2003) used near infrared (NIR) transmittance for the determination
49 of DON in whole wheat kernel samples at levels higher than $500 \mu\text{g kg}^{-1}$, whereas NIR
50 reflectance has been used for detection of scab and estimation of DON and ergosterol in
51 single kernels of highly infected wheat (Dowell et al. 1999). A method for the analysis of
52
53
54
55
56
57
58
59
60

1
2
3
4 *Fusarium* fungi on corn using Fourier transform-mid infrared (FT-MIR) spectroscopy
5
6 with attenuated total reflection (ATR) was developed which enabled the segregation of
7
8 DON contaminated corn samples from non contaminated (blank) samples (Kos et al.
9
10 2003, 2004, 2007; Delwiche and Hareland 2004).
11

12
13
14
15 Papers on the application of FT-IR spectroscopy for a determination of DON content in
16
17 grain mostly exploit the range below 4000 cm^{-1} . In particular, Mossoba et al. (1996)
18
19 reported DON characteristic absorption bands between 1244 and 1750 cm^{-1} . Some
20
21 additional bands at ca. 3500 and close to 3650 cm^{-1} (Young and Games 1994) and not far
22
23 from 1700 cm^{-1} (Abramovic et al. 2007) have been reported. The most recent theoretical
24
25 calculations of IR bands cover the MIR range below 4000 cm^{-1} (Turker and Gumus
26
27 2008), also used by Kos et al. (2007) for corn analysis. Therefore the identification of any
28
29 spectral features of DON within the NIR range is highly desirable. FT-IR spectroscopy
30
31 allows to perform rapid measurements with an excellent signal-to-noise ratio and high
32
33 resolution.
34
35
36
37
38
39
40

41 The aim of the present work was to investigate the feasibility of using FT-NIR for the
42
43 rapid and non-invasive determination of DON in durum and common wheat.
44
45
46
47

48 **Materials and methods**

49
50 *Sample preparation.* A total of 262 wheat (143 durum and 119 common) samples,
51
52 belonging to 32 different varieties, were obtained from wheat naturally infected by
53
54 *Fusarium graminearum* and *Fusarium culmorum* in experimental fields in Northern Italy
55
56
57
58
59
60

1
2
3 during the period from 2002 to 2006. Wheat samples were ground with a Tecator
4 Cyclotec 1093 (International PBI, Hoganas, Sweden) laboratory mill equipped with a 500
5 μm sieve, and were analyzed by HPLC and by FT-NIR spectroscopy. DON levels, as
6 established by HPLC analysis, ranged from 50 to 2600 $\mu\text{g kg}^{-1}$ in durum wheat and from
7 50 to 3000 $\mu\text{g kg}^{-1}$ in common wheat. Samples containing less than 50 $\mu\text{g kg}^{-1}$ DON (the
8 quantification limit of the HPLC-method) were considered as DON-free. Mean
9 recoveries of DON from durum and common wheat samples ($n = 3$) spiked at levels of
10 200, 800 and 1500 $\mu\text{g kg}^{-1}$ were 75-93% with RSD_r of 1.4-14.2% for durum wheat and
11 79-83% with RSD_r of 1.1-12.2% for common wheat. To investigate the distribution of
12 DON during sieving, five durum wheat samples were ground by a Bühler MLI 204
13 (Bühler S.p.A., Milan, Italy) to pass a 1-mm sieve and analyzed by HPLC for DON. A
14 fraction of these samples was sieved to pass a 300 μm sieve, and the two fractions (below
15 and above 300 μm) were analyzed by HPLC.

16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
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 DONTestTM (Vicam, Watertown, MA, USA). After washing the column by passing 5

1
2
3 mL water through it, DON was eluted with 1.5 mL methanol. The eluate was dried under
4
5 nitrogen stream at 50°C, re-dissolved in 200 µL of mobile phase (acetonitrile:water,
6
7 10:90, v/v) and 50 µL were injected onto an LC system with ultraviolet diode array
8
9 detector set to 220 nm.
10
11

12
13
14
15 *FT-NIR analysis.* FT-NIR spectra were recorded using an Antaris II FT-NIR
16
17 spectrophotometer (Thermo Electron Corporation, Madison, WI, USA) equipped with an
18
19 interferometer (containing a fixed and a moving mirror, a beam-splitter), an integrating
20
21 sphere working in diffuse reflection, and an indium and gallium arsenide (InGaAs)
22
23 detector. A sample cup spinner allowed the automatic collection of several subsamples
24
25 from each sample. These subsamples were averaged to obtain representative spectra of
26
27 relatively heterogeneous samples. The integrating sphere's internal reference was also
28
29 used to collect the background spectrum. The moving mirror speed was set at 1.63
30
31 scans/sec. Approximately 30 g of ground wheat samples were placed into the rotating
32
33 sample cup spinner with a quartz cup. Spectroscopic data were recorded as absorbance
34
35 between 10000-4000 cm⁻¹ with 8 cm⁻¹ resolution to give a total of 750 points per sample.
36
37 Each sample spectrum, consisting of an average of 128 consecutive interferometer scans,
38
39 was collected in about 1 min 20 s and stored in a separate data file.
40
41
42
43
44
45
46
47

48 FT-NIR transmission spectra of DON were obtained using the Transmission module
49
50 (Thermo Electron Corporation) consisting of a sample holder to allocate glass tubes.
51
52 Approximately 1000 µl of solution were transferred into glass culture tubes (6 x 50 mm).
53
54 Spectroscopic data were recorded as absorbance between 10000-4000 cm⁻¹ with 8 cm⁻¹
55
56
57
58
59
60

1
2
3 resolution and each sample spectrum consisted of an average of 16 consecutive
4
5 interferometer scans.
6
7

8
9
10 Commercially available crystalline DON standard (Sigma, Milan, Italy) was dissolved in
11
12 acetonitrile to prepare a 1 mg ml^{-1} standard solution. FT-NIR transmission spectrum of
13
14 DON was determined after subtraction of the mean spectrum ($n = 10$) of acetonitrile from
15
16 the mean spectrum ($n = 10$) of DON standard solution. Furthermore, two sub-samples of
17
18 a DON-free durum wheat sample ($< 50 \text{ } \mu\text{g kg}^{-1}$) were spiked with acetonitrile and with
19
20 the same volume of DON standard solution (spiking level $10000 \text{ } \mu\text{g kg}^{-1}$), respectively.
21
22 The FT-NIR spectrum was obtained after subtraction of the spectrum of acetonitrile
23
24 spiked wheat from that of DON spiked wheat. Similarly, another FT-NIR spectrum was
25
26 obtained after subtraction of a DON-free durum wheat spectrum from that of a naturally
27
28 contaminated one ($13000 \text{ } \mu\text{g kg}^{-1}$ DON).
29
30
31
32
33
34
35

36 *Multivariate data analysis.* Based on DON content (as determined by HPLC analysis)
37
38 wheat samples were distributed into classes each covering $100 \text{ } \mu\text{g kg}^{-1}$ DON. Calibration
39
40 and validation samples were randomly selected from each class by considering an
41
42 approximate ratio of 2.5:1 between calibration and validation samples. Spectroscopic data
43
44 were processed by TQ Analyst[®] professional edition (Thermo Electron Corporation). The
45
46 Spectrum Outlier diagnostic (TQ Analyst[®]) was run to seek sample spectra that were the
47
48 most different from the other samples. In particular, it was calculated the mean spectrum
49
50 for all calibration samples, measured the distance between the mean spectrum and the
51
52 spectrum of each calibration sample. The Chauvenet Test estimated whether the
53
54
55
56
57
58
59
60

1
2
3 difference between spectra was significant. Once the initial population was free of
4 spectral outliers calibration models were developed and statistically elaborated for
5
6 quantitative analysis using PLS regression with full cross validation (*leave one out*).
7
8 DON data obtained by FT-NIR were transformed using Standard Normal Variate (SNV)
9
10 (Barnes et al., 1989), first derivative, mean centering and Savitzky-Golay smoothing (2nd-
11
12 order polynomial filtering operation performed on 3 points) (Savitzky and Golay, 1964),
13
14 and correlated with those obtained by HPLC.
15
16
17
18
19
20
21

22 The Partial Least Squares (PLS) models performance were evaluated by calculating the
23 correlation coefficient (*r*) between the reference (HPLC data) and the predicted (FT-NIR
24 data) DON levels, the root mean square error of calibration (RMSEC), the root mean
25 square error of cross-validation (RMSECV) and the number of PLS factors. External data
26 sets (validation sets) were also used to evaluate the performance of the models for
27 prediction, and the root mean square error of prediction (RMSEP) values were calculated.
28
29 Validation sets were selected to cover the range of DON-content of interest. RMSEC,
30
31 RMSECV and RMSEP were expressed by the same DON level units ($\mu\text{g kg}^{-1}$) in wheat
32 samples. In the development of all calibration models, 10 PLS factors were set up as a
33 maximum number to work with. The optimum number of PLS factors was automatically
34 calculated by the TQ Analyst[®] software corresponding to the lowest number of factors
35 giving the closest to minimum value of the prediction residual error sum of squares
36 (PRESS). As the calculated number of PLS factors gave models that strictly dependent on
37 calibration samples, an appropriate number was chosen giving comparable RMSEC,
38
39 RMSECV and RMSEP values.
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 The accuracy of each model was evaluated based on the coefficient of determination (r^2)
4 for predicted *versus* reference DON levels in calibration and validation sets. It was
5 assumed that with $r^2 = 0.50-0.65$ discrimination between high and low values was
6 possible, $r^2 = 0.66-0.81$ allowed approximate quantitative prediction, $r^2 = 0.82-0.90$
7 allowed good prediction, and excellent calibration model were obtained with $r^2 = 0.91$ or
8 higher (Williams 2003). The prediction accuracy of models was also evaluated based on
9 the residual predictive deviation (RPD), defined as the ratio of the standard deviation of
10 the reference DON values in the validation set to the RMSEP. In general, RPD values of
11 3.1-4.9 indicate NIR calibration models suitable for screening purposes, whereas values
12 of 5.0-6.4 are considered adequate for quality control (Fearn 2002). However, a more
13 qualitative interpretation of the RPD considers values lower than 1.5 insufficient for most
14 applications and values greater than 2 as excellent (Williams 2001; Smyth et al. 2008).

15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32 The range error ratio (RER), calculated by dividing the range of the reference DON
33 values in the validation set by the RMSEP, was used as a useful indicator to assess the
34 practical utility of the calibration as a predictive model. Models with RER of less than 3
35 had a little practical utility; between 3 and 10 had limited to good practical utility; and
36 above 10 indicated a high practical utility value (Fearn 2002; Williams 2003).

37
38
39
40
41
42
43
44
45
46 *Qualitative analysis of different wheat cultivars.* The ability of FT-NIR spectroscopy to
47 discriminate between blank and naturally contaminated samples of different cultivars of
48 both durum and common wheat was evaluated by Discriminant Analysis. Based on the
49 DON content as determined by HPLC analysis, samples were split into two classes, one
50 including samples with less than $300 \mu\text{g kg}^{-1}$ DON (assumed as “blank”) and the other
51
52
53
54
55
56
57
58
59
60

1
2
3 one including naturally contaminated (from 300 $\mu\text{g kg}^{-1}$ up to about 3000 $\mu\text{g kg}^{-1}$ DON)
4
5 wheat samples. Validation samples were randomly selected to cover the range of interest
6
7 (up to 2000 $\mu\text{g kg}^{-1}$). Calibration and validation sets contained samples in the
8
9 approximate ratio of 3:1. All spectroscopic data were transformed using variance scaling
10
11 normalization, Standard Normal Variate (SNV) (Barnes et al., 1989), first derivative,
12
13 mean centering and Savitzky-Golay smoothing (2nd-order polynomial filtering operation
14
15 performed on 5 points) (Savitzky and Golay, 1964).
16
17
18
19
20
21

22 Discriminant Analysis was performed to create the distribution model by using the
23
24 spectral information from all calibration samples. In particular, during calibration, the
25
26 software computed an average spectrum and then generated a distribution model by
27
28 estimating the variance at each frequency in the analysis range. When the calibrated
29
30 method was used to analyze validation samples, the software performed a Principal
31
32 Component Analysis (PCA) on the validation sample spectra and on the variance spectra
33
34 to determine score values. The scores were used to produce Mahalanobis distance values,
35
36 which in turn were used to rank the classes. Discriminant Analysis assigned the ranking
37
38 class based on the level of similarity with the spectrum of the validation sample. The
39
40 Mahalanobis distance between the validation sample and each class center was also
41
42 recorded.
43
44
45
46
47
48
49
50

51 **Results and discussion**

52
53 *Effect of sample preparation on DON analysis by FT-NIR analysis.* Kos et al. (2003;
54
55 2007) reported that the repeatability of spectral measurements performed on ground
56
57
58
59
60

1
2
3 maize (particle size < 1 mm) was not good enough for attempting classification by MIR-
4
5 ATR of DON contaminated maize, and that a sieving procedure (selecting particles with
6
7 diameter between 250 and 100 μm) improved the repeatability of spectral measurements
8
9 from 20% to 4.4%.
10
11

12
13
14 As an initial approach, ground wheat samples were sieved to pass a 300 μm sieve and
15
16 analysed by FT-NIR spectroscopy. An alternative sample preparation was used to avoid
17
18 the time-consuming sieving step (20-30 min if carried out after grinding). In particular,
19
20 wheat samples were milled with a Tecator Cyclotec laboratory mill (Tecator Cyclotec)
21
22 equipped with a 500 μm sieve and the obtained fraction was directly analysed by FT-NIR
23
24 spectroscopy. Spectra for 50 repetitive measurements of a sieved sample were compared
25
26 to those of an unsieved sample of the same wheat variety and with a comparable DON-
27
28 content.
29
30
31
32
33
34
35

36
37 In order to statistically evaluate spectra differences between wheat samples with particle
38
39 size < 300 μm and < 500 μm , the analysis of variance (ANOVA, one way) was applied to
40
41 the two groups of repetitive measurements. No significant difference was found within
42
43 each group, while a significant difference ($F = 29.62$, $F_{\text{critic}} = 3.84$) was observed between
44
45 the mean spectra of the two groups due to the different distribution of particle sizes and
46
47 relevant chemical composition. The PCA score/score plot revealed that the cluster
48
49 containing samples with particle size < 300 μm was more scattered than the cluster
50
51 containing samples with particle size < 500 μm , showing a more homogeneous
52
53 distribution of particle size in the unsieved sample (Figure 1).
54
55
56
57
58
59
60

[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 $> 300 \mu\text{m}$, and accumulated by 115% in fractions with particle size $< 300 \mu\text{m}$.

[Insert Table I about here]

Consequently, the sample preparation approach performed by using a Tecator Cyclotec milling at $< 500 \mu\text{m}$ 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

1
2
3 band at 7000 cm^{-1} was due to an overlapping of DON and water absorption bands. The
4
5 same absorption bands were observed in the FT-NIR spectrum obtained after subtraction
6
7 of a DON-free wheat spectrum from that of a naturally contaminated one (Figure 2, line
8
9 c).
10
11

12
13
14
15 [Insert Figure 2 about here]
16
17
18
19

20 DON levels in wheat are generally several orders of magnitude lower than levels of the
21
22 main wheat constituents (protein, starch, etc.), and spectra recorded for contaminated and
23
24 uncontaminated wheat may differ one from another more because of difference in
25
26 chemical composition (due to development of the fungus) than because of DON content.
27
28 Thus, subtraction of the spectra can produce spurious features not necessarily related to
29
30 DON content due to the relatively low DON levels commonly occurring in wheat.
31
32 However, the use of highly contaminated wheat (at levels higher than $10000\text{ }\mu\text{g kg}^{-1}$)
33
34 produced subtractive spectra with appreciable FT-NIR spectral bands (particularly at
35
36 5251 cm^{-1}) related to DON as shown in Figure 3. The characteristic absorption bands of
37
38 DON could not be identified in wheat samples contaminated at lower levels, such as
39
40 those used herein (up to $3000\text{ }\mu\text{g kg}^{-1}$) for the three PLS regression models. Nevertheless,
41
42 a certain discrimination between low and high DON levels was obtained by considering
43
44 the entire spectral range between 10000 and 4000 cm^{-1} (see below).
45
46
47
48
49
50

51
52
53 *DON analysis by FT-NIR spectroscopy.* Three PLS regression models were built by
54
55 considering the spectral range between 10000 and 4000 cm^{-1} for durum, common and
56
57
58
59
60

1
2
3 durum+common wheat samples, respectively. Table II summarizes sample statistics for
4
5 the calibration and validation set of durum, common and durum+common wheat.
6
7 Comparing the mean DON levels (ranging from 439 to 887 $\mu\text{g kg}^{-1}$) with median levels
8
9 (from 188 to 656 $\mu\text{g kg}^{-1}$) for durum or common wheat samples, a very skewed data
10
11 distribution was observed, with many small values and fewer large ones. After
12
13 combination of the two wheat species (durum+common wheat) a better distribution of
14
15 DON level was obtained and the resulting mean (585-750 $\mu\text{g kg}^{-1}$) and median (395-523
16
17 $\mu\text{g kg}^{-1}$) DON levels were closer.
18
19
20
21

22
23
24 [Insert Table II about here]
25
26
27

28
29 Validation sets of common and durum wheat were selected, both consisting of 30
30
31 samples. The two PLS models were validated up to about 2000 $\mu\text{g kg}^{-1}$ DON to cover the
32
33 range of regulatory levels set by the EU for unprocessed wheat and most wheat based
34
35 products. The selection was performed based on DON content (measured by HPLC) in
36
37 order to have as many samples as possible of the validation set within the range of DON
38
39 content to be considered for the calibration (Table II).
40
41
42
43
44

45
46 Running the PLS analysis on the calibration sets a positive linear correlation ($r > 0.90$)
47
48 was obtained in the measured range for both models, indicating a good fit between HPLC
49
50 and FT-NIR data. According to the coefficients of determination (r^2) and slope values,
51
52 both > 0.82 , a good prediction of DON content by FT-NIR spectroscopy was achieved in
53
54 both PLS calibration models (Figure 3a and 3b).
55
56
57
58
59
60

[Insert Figure 3 about here]

Furthermore, RMSECV values were 470 and 516 $\mu\text{g kg}^{-1}$ 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 $\mu\text{g kg}^{-1}$ for durum wheat and 348 $\mu\text{g kg}^{-1}$ 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

1
2
3 model to discriminate between high and low contaminated samples. However, it is
4
5 evident that a further improvement of RPD values, and then more accurate PLS models,
6
7 could be obtained by increasing the range and the distribution of DON content in the
8
9 validation samples. On the other hand, RER values lie within 6 and 7, indicating a good
10
11 practical utility of the models for DON prediction in wheat samples (Table III).
12
13

14
15 Results obtained in the present study were similar to those reported by Pettersson and
16
17 Aberg (2003) in terms of correlation coefficient, slope and RMSEP. However, a
18
19 remarkable improvement was obtained with the PLS regression models used herein for
20
21 prediction of DON in durum and common wheat because calibrations were made on a
22
23 larger number of calibration and validation samples. Furthermore DON contamination
24
25 levels used herein were close to the DON maximum limits set by the European Union for
26
27 unprocessed durum ($1750 \mu\text{g kg}^{-1}$) and common ($1250 \mu\text{g kg}^{-1}$) wheat, whereas
28
29 Pettersson and Aberg (2003) calibrated the models at higher and less realistic levels (up
30
31 to $10000\text{-}15000 \mu\text{g kg}^{-1}$).
32
33
34
35
36
37
38

39 An additional PLS calibration model was built by combining durum and common wheat
40
41 samples to evaluate the possibility to include different wheat species belonging to
42
43 different varieties in the same model. Also in this case a good fit of the PLS model ($r =$
44
45 0.84) was obtained (Figure 3, Table III). The r^2 values indicated that the model
46
47 approximated quantitative predictions of DON in the calibration set ($r^2 = 0.71$) and
48
49 allowed for discrimination between high and low DON levels in the validation set ($r^2 =$
50
51 0.58) (Table III). These results were also confirmed by the RER value (6.3), while the
52
53 RPD value (1.0) failed to attain the minimum recommended for quantitative analysis.
54
55
56
57
58
59
60

1
2
3 Although results obtained in the present study are not fully satisfying for quantitative or
4 semi-quantitative analysis, they provide a good evidence of the feasibility of using FT-
5 NIR spectroscopy as a screening tool for determination of DON in wheat samples.
6
7
8 Furthermore, considering that different wheat species (durum and common) and wheat
9 varieties contain different amount of chemical constituents, such as protein, lipids and
10 moisture with remarkable spectral differences (Cocchi et al., 2006; Morris et al., 2005),
11
12 our findings indicate that this variability does not affect the applicability of FT-NIR for
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 $\mu\text{g kg}^{-1}$ DON) and the other one including 113 “contaminated” samples ($> 300 \mu\text{g kg}^{-1}$ 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,

1
2
3 respectively. The boundaries, displayed as perpendicular lines in the plot, show the 95%
4
5 confidence intervals for the two classes. All calibration and validation samples relevant to
6
7 the “blank” class are expected to be clustered in the upper left corner of the plot, while
8
9 samples falling in the lower right corner define the “contaminated” class. As observed in
10
11 Figure 4, a few calibration samples of the two classes fall in the uncertainty area (lower
12
13 left corner, 40 out of 197). Most of them were “blank” (16%) or contained DON levels
14
15 between 300 and 500 $\mu\text{g kg}^{-1}$ (30%), indicating that the model had a limited degree of
16
17 specificity for wheat samples contaminated with DON levels close to the cut-off limit.
18
19 When the obtained model was applied to a set of 65 validation samples, it correctly
20
21 classified 69% of samples as “blank” or “contaminated” wheat. The remaining 20
22
23 samples were incorrectly classified as false positive or negative sample. Also in this case,
24
25 most of misclassified samples were “blank” (55%) or contained DON levels ranging from
26
27 300 to 400 $\mu\text{g kg}^{-1}$ (25%), confirming that the goodness of the discrimination model was
28
29 greatly influenced by the low DON cut-off limit fixed to distinguish the two classes.
30
31 Lower cut-off levels, i.e. 50 $\mu\text{g kg}^{-1}$ (the quantification limit of the HPLC method), 100
32
33 and 200 $\mu\text{g kg}^{-1}$, led to lesser precise models, while higher cut-off levels gave poorly
34
35 reliable models due to problems associated with the relatively limited number of samples
36
37 with high DON levels. However, these results still recommend the use of FT-NIR as a
38
39 sorting tool for screening purpose, since the legal limit at present is well over 300 $\mu\text{g kg}^{-1}$
40
41 for most commodities (with the important exception of baby food where the limit is 200
42
43 $\mu\text{g kg}^{-1}$). An improvement to this qualitative model could be obtained by increasing the
44
45 number of samples, and in particular, by increasing the number of high DON
46
47 contaminated samples.
48
49
50
51
52
53
54
55
56
57
58
59
60

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

Acknowledgments

We are grateful to Dr. Tom Fearn, Department of Statistical Science, University College London, UK, for his advices on statistical elaboration of FT-NIR data.

This work was supported in part by the National Research Council, Italy, the Short-Term Mobility Program 2007.

References

- 1
2
3 Abramovic B, Jajic I, Abramovic B, Cosic J, Juric V. 2007. Detection of deoxynivalenol
4 in wheat by Fourier Transform Infrared Spectroscopy. *Acta Chimica Slovenica*
5
6 54:859-867.
7
8
9
10 Barnes BJ, Dhanoa MS, Lister SJ. 1989. Standard Normal Variate Transformation and
11
12 De-trending of Near-Infrared Diffuse Reflectance Spectra. *Applied Spectroscopy*
13
14 43:772-777.
15
16
17 Canady RA, Coker RD, Egan SK, Krska R, Kuiper-Goodman T, Olsen M, Pestka J,
18
19 Resnik S, Schlatter J. 2001. Deoxynivalenol. In: Safety evaluation of certain
20
21 mycotoxins in food. Fifty-sixth Meeting of the Joint FAO/WHO Expert Committee
22
23 on Food Additives (JECFA), FAO Food and Nutrition paper 74, WHO Food
24
25 Additives ser. 47. World Health Organization, Geneva. p 419.
26
27
28 Cocchi M, Durante C, Foca G, Marchetti A, Tassi L, Ulrici U. 2006. Durum wheat
29
30 adulteration detection by NIR spectroscopy multivariate calibration. *Talanta*
31
32 68:1505-1511.
33
34
35
36 Delwiche SR, Hareland GA. 2004. Detection of scab-damaged hard red spring wheat
37
38 kernels by near-infrared reflectance. *Cereal Chemistry* 81:643-649.
39
40
41 Dowell FE, Ram MS, Seitz MS. 1999. Predicting scab, vomitoxin, and ergosterol in
42
43 single wheat kernels using near-infrared spectroscopy. *Cereal Chemistry* 76:573-576.
44
45
46 European Commission (2007). Commission regulation (EC) No 1126/2007 of 28
47
48 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels
49
50 for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize
51
52 products. *Official Journal of European Union*. L 255:14-17.
53
54
55 Fearn T. 2002. Assessing calibrations: SEP, RPD, RER and R^2 . *NIR news* 13:12-14.
56
57
58
59
60

- 1
2
3 Kos G, Lohninger H, Krska R. 2003. Development of a method for the determination of
4 *Fusarium* fungi on corn using mid-infrared spectroscopy with attenuated total
5 reflection and chemometrics. *Analytical Chemistry* 75:1211-1217.
6
7
8
9
10 Kos G, Krska R, Lohninger H, Griffiths PR. 2004. A comparative study of mid-infrared
11 diffuse reflection (DR) and attenuated total reflection (ATR) spectroscopy for the
12 detection of fungal infection on RWA2-corn. *Analytical and Bioanalytical Chemistry*
13 378:159-166.
14
15
16
17
18
19
20 Kos G, Lohninger H, Mizaikoff B, Krska R. 2007. Optimisation of a sample preparation
21 procedure for the screening of fungal infection and assessment of deoxynivalenol
22 content in maize using mid-infrared attenuated total reflection spectroscopy. *Food*
23 *Additives and Contaminants* 24 (7):721-729.
24
25
26
27
28
29 Lippolis V, Pascale M, Visconti A. 2006. Optimization of a fluorescence polarization
30 immunoassay for rapid quantification of deoxynivalenol in durum wheat-based
31 products. *Journal of Food Protection* 69:2712-2719.
32
33
34
35
36
37 Morris CF, Campbell KG, King GE. 2005. Kernel texture differences among US soft
38 wheat cultivars. *Journal of the Science of Food and Agriculture* 85 (11):1959-1965.
39
40
41 Mossoba MM, Adams S, Roach JAG, Trucksess MW. 1996. Analysis of trichothecene
42 mycotoxins in contaminated grains by gas chromatography/matrix isolation/Fourier
43 transform infrared spectroscopy and gas chromatography/mass spectrometry. *Journal*
44 *of AOAC International* 75 (5):1116-1123.
45
46
47
48
49
50 Mouazen AM, De Baerdemaeker J, Ramon H. 2005. Towards development of on-line
51 soil moisture content sensor using a fibre-type NIR spectrophotometer. *Soil Tillage*
52 *Research* 80:171-183.
53
54
55
56
57
58
59
60

- 1
2
3 Pettersson H, Aberg L. 2003. Near infrared spectroscopy for determination of mycotoxins
4
5 in cereals. *Food Control* 14: 229-232.
6
7
8 Savitzky A, Golay MJE. 1964. Smoothing and differentiation of data by simplified least
9
10 squares procedures. *Analytical Chemistry* 36:1627-1639.
11
12
13 SCF (Scientific Committee on Food) 2002. Opinion of the Scientific Committee on Food
14
15 on *Fusarium* toxins. Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and
16
17 deoxynivalenol, adopted on 26 February 2002. European Commission. Available
18
19 from: http://europa.eu.int/comm/food/fs/sc/scf/out123_en.pdf.
20
21
22 Schlatter J. 2004. Toxicity data relevant for hazard characterization. *Toxicology Letters*
23
24 153:83-89.
25
26
27 Schothorst R, van Egmond H. 2004. Report from Scoop task 3.2.10. Collection of
28
29 occurrence data of *Fusarium* toxins in food and assessment of dietary intake by the
30
31 population of EU members states. Subtask: trichothecenes. *Toxicology Letters*
32
33 153:133-143.
34
35
36 Smyth HE, Cozzolino D, Cynkar WU, Damberg RG, Sefton M, Gishen M. 2008. Near
37
38 infrared spectroscopy as a rapid tool to measure volatile aroma compounds in
39
40 Riesling wine: possibilities and limits. *Analytical and Bioanalytical Chemistry*
41
42 390:1911-1916.
43
44
45
46 Turker L, Gumus S. 2008. A theoretical study on vomitoxin and its tautomers. *Journal of*
47
48 *Hazardous Material*, Article in Press. DOI: 10.1016/j.jhazmat.2008.06.087
49
50
51 Young GC, Games DE. 1994. Analysis of *Fusarium* mycotoxins by gas chromatography-
52
53 Fourier transform infrared spectroscopy. *Journal of Chromatography*, 663(2): 211-
54
55 218.
56
57
58
59
60

1
2
3 Williams, PC. (2001). Implementation of near-infrared technology. In Near-infrared
4 technology in the agricultural and food industries. 2nd edition, P. Williams & K.
5
6 Norris (Eds.), St. Paul, MN: American Association of Cereal Chemists. p. 145.
7
8

9
10 Williams, P. 2003. Near-infrared technology-Getting the best out of light. In: A short
11 course in the practical implementation of near infrared spectroscopy for the user. 1.1
12
13 PDK Projects Inc., editors. Nanaimo, Canada. p 109.
14
15

16
17 Visconti A, Haidukowski EM, Pascale M, Silvestri M. 2004. Reduction of
18 deoxynivalenol during durum wheat processing and spaghetti cooking. Toxicology
19
20 Letters 153
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2 **Figure 1.** Score/score plot of spectral data (PC1 vs PC2) from 50 repetitive measurements of
3
4 two wheat samples with different particle sizes. The two clusters represent wheat samples
5
6 with particle size < 500 μm (left, unsieved) and < 300 μm (right, sieved).
7

8
9 **Figure 2.** FT-NIR subtractive spectra of deoxynivalenol in acetonitrile spectrum from that of
10 acetonitrile (line a), of acetonitrile spiked wheat spectrum from that of DON spiked (10000
11 $\mu\text{g kg}^{-1}$) wheat (line b) and of deoxynivalenol-free wheat spectrum from that of naturally
12 contaminated (13000 $\mu\text{g kg}^{-1}$) wheat.
13
14
15
16

Deleted: of pure commercially available crystalline

Deleted: lower

Deleted: a

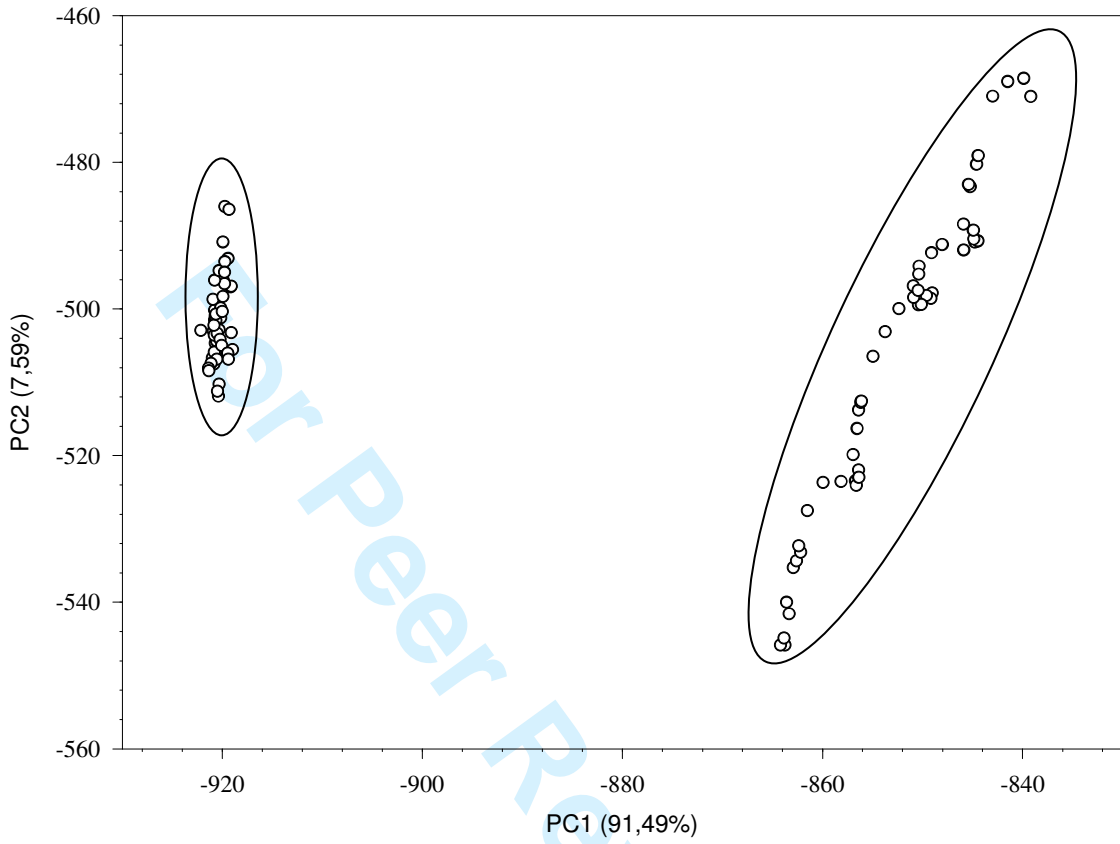
Deleted: wheat sample after subtraction of a blank wheat spectrum (upper line).

Deleted:

17
18 **Figure 3.** PLS regression models of deoxynivalenol content determined by FT-NIR and the
19 reference method (HPLC) of durum (3 a), common (3 b) and durum+common (3 c) wheat
20 samples.
21
22
23

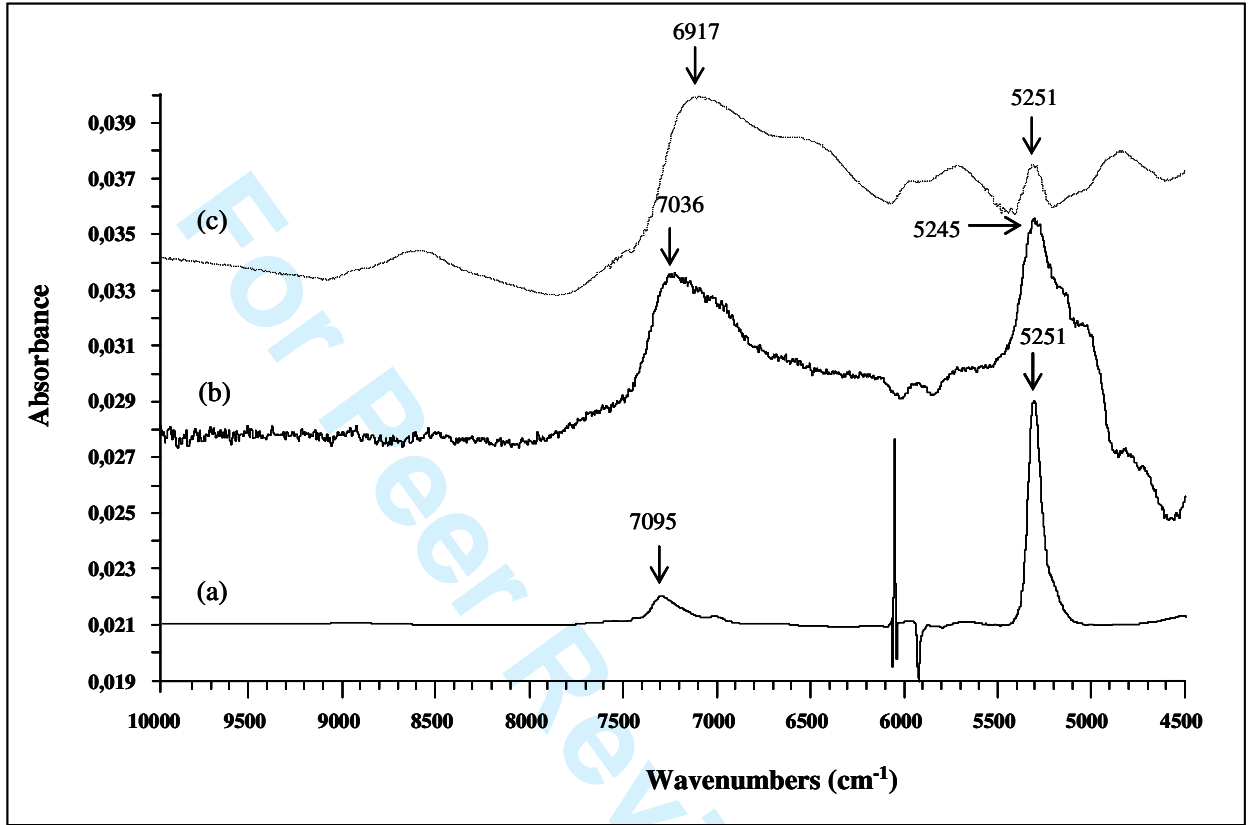
24
25
26 **Figure 4.** Pairwise distance plot reporting the Mahalanobis distance between each sample and
27 the "Blank" and "Contaminated" classes.
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 **Figure 1.**

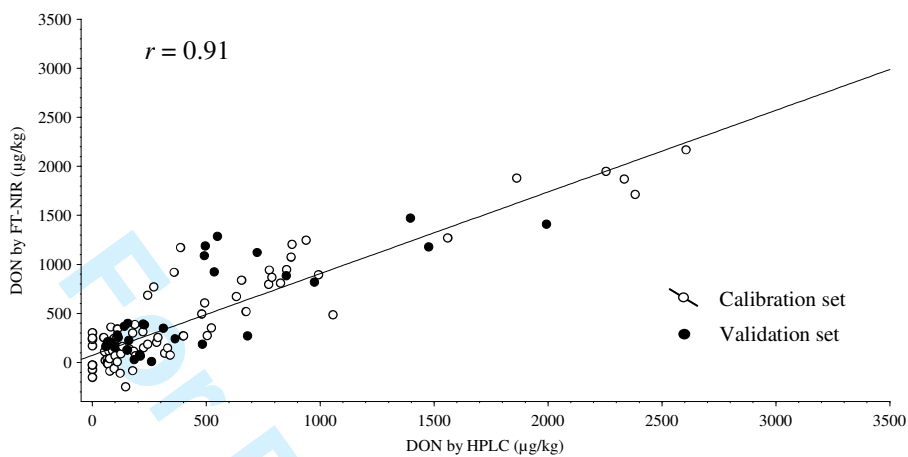


2
3
4

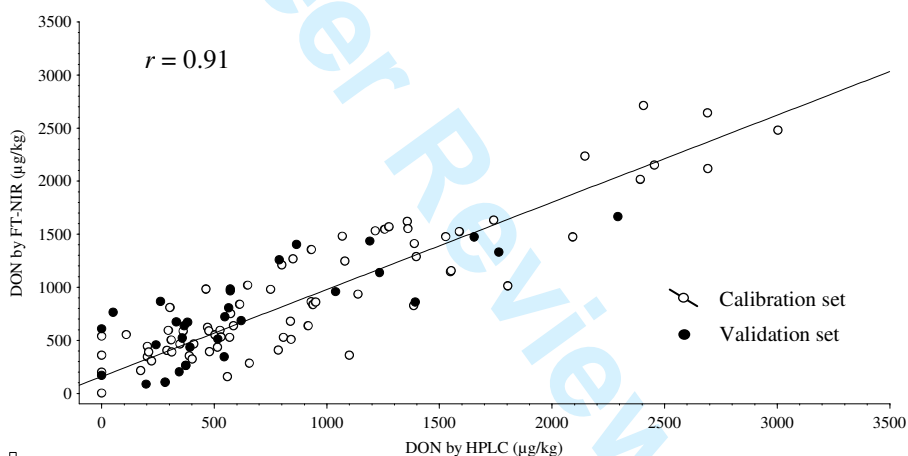
1 **Figure 2.**



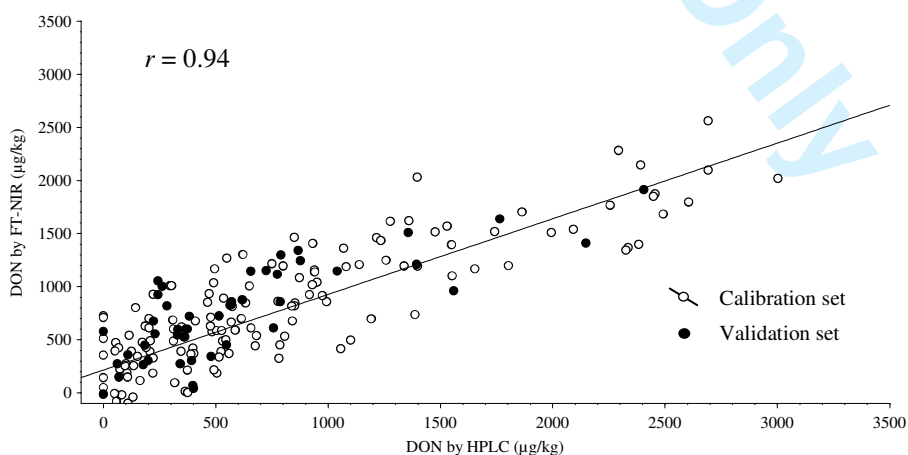
1 **Figure 3 (a)**



23 **Figure 3 (b)**

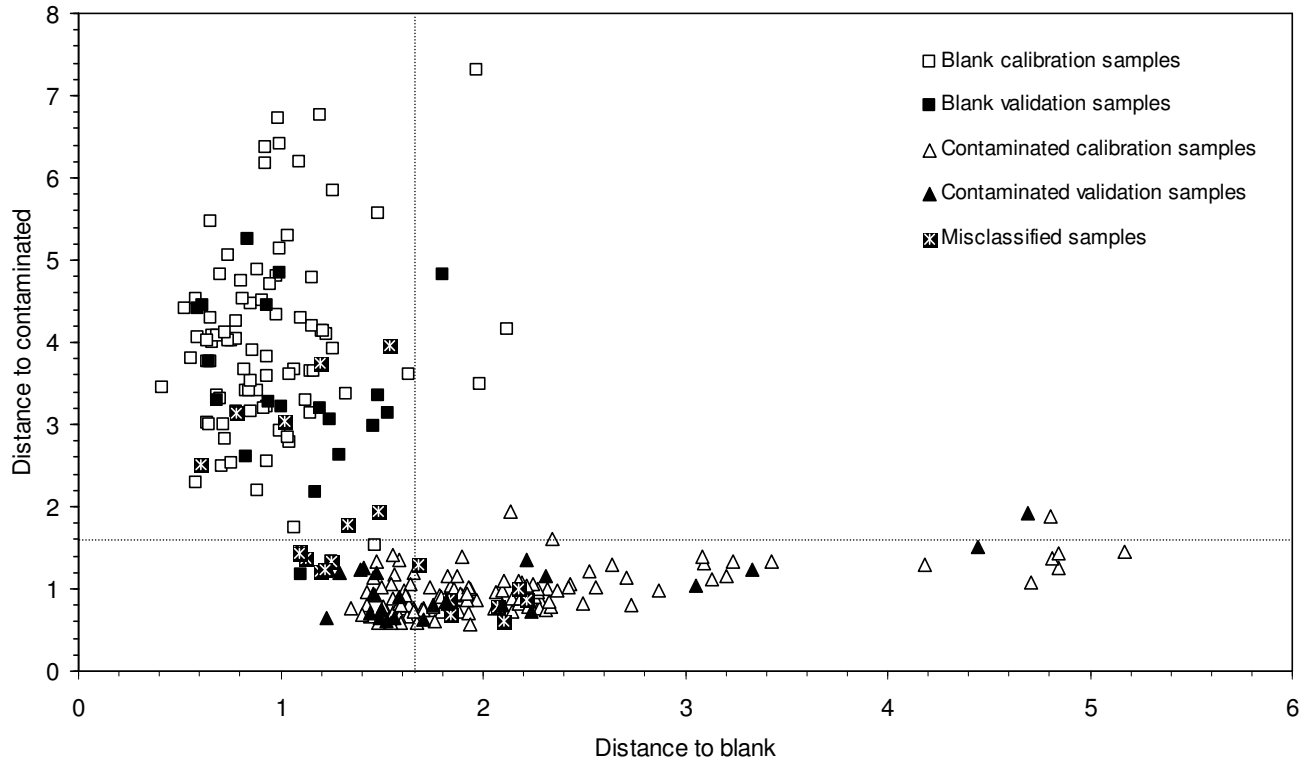


42 **Figure 3 (c)**



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 **Figure 4.**



view Only

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

Wheat sample (#)	Unsieved ($\mu\text{g}/\text{kg}$)	Sieved, < 300 μm ($\mu\text{g}/\text{kg}$)	Recovery (%)*	Sieved, > 300 μm ($\mu\text{g}/\text{kg}$)	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 \pm SD			115 \pm 8	84 \pm 9	

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

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 ($\mu\text{g}/\text{kg}$)	0-2600	0-3000	0-3000	60-1990	0-2290	0-2400
Median ($\mu\text{g}/\text{kg}$)	188	656	523	245	530	395
Mean ($\mu\text{g}/\text{kg}$)	439	887	750	459	658	585
SD* ($\mu\text{g}/\text{kg}$)	590	720	722	470	681	533

*SD = standard deviation

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
$r_{\text{calibration}}$	<u>0.91</u>	<u>0.91</u>	<u>0.84</u>
$r^2_{\text{calibration}}$	0.83	0.82	0.71
Slope _{validation}	0.79	0.62	0.71
$r_{\text{validation}}$	<u>0.80</u>	<u>0.79</u>	<u>0.76</u>
$r^2_{\text{validation}}$	0.62	0.63	0.58
RMSEP ($\mu\text{g}/\text{kg}$)	306	348	379
RMSECV ($\mu\text{g}/\text{kg}$)	470	516	555
PLS factors	4	4	5
RER	6.3	6.6	6.3
RPD	1.6	2.0	1.0

Deleted: RMSEC ($\mu\text{g}/\text{kg}$) ... [1]

Deleted: RMSEC, root mean square error of calibration set;

* 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.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Page 1: [1] Deleted	DeGirolamo	11/27/2008 6:46:00 PM	
RMSEC ($\mu\text{g}/\text{kg}$)	240	303	386

For Peer Review Only