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Near-infrared spectroscopy as a tool for rapid screening of deoxynivalenol in wheat flour and its applicability in the industry

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ABSTRACT

This study aimed to evaluate the applicability and efficiency of Near-Infrared Spectroscopy (NIR) by using dispersive NIR and Fourier Transform NIR to analyse 267 samples of Brazilian wheat flour contaminated with deoxynivalenol (DON). For this, Partial Least-squares Discriminant Analysis (PLS-DA) and Principal Component Analysis-Linear Discriminant Analysis (PC-LDA) were used as discriminatory methods. Next, the samples were classified according to the maximum tolerated limits (MTL) for DON in Brazil, 750 $\mu\text{g kg}^{-1}$, and two groups were established for the calibration set: category A ($\leq 450 \mu\text{g kg}^{-1}$), non-contaminated or below the MTL; and category B ($> 450 \mu\text{g kg}^{-1}$), contaminated or above the MTL. Validation samples through PLS-DA showed correct classification rates in the range of 85–87.5% and presented a 10–15% error; for PC-LDA, the hit rate was over 85% with an error of 10–15%. The present findings demonstrate that NIR is an excellent alternative method to classify wheat flour samples according to DON content.

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Food safety; mycotoxins; infrared spectroscopy; screening; classification methods; Brazil

Introduction

Wheat (*Triticum spp.*) is the third most cultivated food crop worldwide, after maize and rice. About 65% of its total production is destined for human dietary intake, and 17% and 12% are used in animal feed and industrial applications, respectively (FAO 2020). In Brazil, wheat production reached approximately 5.2 million tons in 2019; winter wheat produces the best yields, and the southern states of Paraná and Rio Grande do Sul account for 88% of the national production (IBGE 2020). Nevertheless, the domestic demand (about 11 million tons) is much greater than the annual national yield (ABITRIGO 2020), hence the need to import from countries such as Argentina, the United States and Paraguay (ABITRIGO 2020).

Quality of wheat grains interacts with the characteristics of final products. Aspects related to cultivation and harvesting conditions, efficient drying, and proper storage must be carefully controlled along the production chain in order to avoid grain damage and the onset of diseases (Magan et al. 2010). *Fusarium* head blight (FHB) or scab is one of the most important diseases occurring in wheat,

and its causative agent is the fungus *Fusarium graminearum* (Mendes et al. 2018). Low temperatures, heavy rains, and high humidity are the climatic conditions which favour the development of this pathogen and consequent production of mycotoxins, thus lowering the quality of the cereal (Prandini et al. 2009).

Deoxynivalenol (DON) is the most prevalent mycotoxin in wheat. It is one of the chief toxins produced by certain *Fusarium* species that frequently occur in wheat and other grains either in the field or during storage. DON is a trichothecene which belongs to group B; this polar organic compound is a tetracyclic sesquiterpene chemically named as 12,13-epoxy-3,4,15-trihydroxytrichothec-9-en-8-one, (3 α ,7 α)-(9 CI) (Nagy et al. 2005). The toxic activity of DON is associated with the presence of three free hydroxyl groups (-OH) in its molecular structure (Sobrova et al. 2010).

The occurrence of DON in ingredients used to produce food and feed has a well-known toxicological impact. In humans, there are reports of outbreaks in Asia associated with DON intoxication with symptoms of gastrointestinal disorder, nausea,

vomiting, and diarrhoea (Bhat et al. 1989). DON is found in cereals used to produce items which are highly consumed by the general population, such as bread, cake, biscuit, pasta, and pizza, hence the concern over its occurrence (Vidal et al. 2018).

Trichothecenes are relatively stable at high temperatures (up to 120°C). The content of DON can be reduced after food processing, but the mycotoxin cannot be completely destroyed (Voss and Snook 2010; Krska et al. 2016; Suman and Generotti 2016; Guo et al. 2020). Therefore, many countries have stipulated maximum tolerated limits (MTL) to control their products and minimise the risks of contamination. In Brazil, the National Health Surveillance Agency (ANVISA) regulates the MTL for mycotoxins in food (ANVISA 2011). ANVISA has made the MTL for wheat flour increasingly strict over the years: 1,750 in 2012, 1,250 in 2014, 1,000 in 2017, and 750 $\mu\text{g kg}^{-1}$ in 2019.

In light of the above, mycotoxicological monitoring via analysis of wheat flour is essential. Traditional methods involve extracting the toxin, cleanup, then chromatography (Lino et al. 2006); despite being very sensitive, these methods are time-consuming, sample-destructive, and costly, thus hindering large-scale screening (Tao et al. 2019). Numerous methods widely used in routine analysis of mycotoxin are based on the ELISA test (Enzyme-Linked Immunosorbent Assay), which is an immunoenzymatic technique. It is commonly applied for mycotoxin detection due to its speed, ease of use, specificity and sensitivity, being capable of detecting low concentrations of mycotoxins (Zheng et al. 2006). Optical methods have also been gaining ground in the industry. Their principle is the absorption of infrared light by organic compounds, mainly CH, OH, NH (Morgano et al. 2005). Among the multiple benefits which justify the growing use of such techniques are: non-destructive analyses; various samples may be analysed together; and prompt availability of results, thus expediting decision-making (Lim et al. 2018).

Dispersive and FT-NIR instruments have been employed in the cereal industry to determine attributes related to quality and the presence of mycotoxins (Fernández-Ibañez et al. 2009; Pandey et al. 2018). When cereals are attacked by fungus and subsequent contamination occurs, there is a change

in the chemical structure (protein and carbohydrates) and the food spectra (Shi and Yu 2018). Such spectral alterations require the use of multivariate analysis tools, and the information contained in the various wavelengths can be examined and used to build quantitative or classification models.

A number of papers have tested NIR technology to analyse mycotoxins in wheat, especially DON. Peiris et al. (2009) examined the NIR absorbance characteristics (range: 350–2500 nm) of wheat kernels with (artificial inoculation) or without DON; the authors identified absorption bands at 1408, 1904, and 1909 nm, and observed differences at 1204, 1365, and 1700 nm, which were attributed to variations in the content of food reserves as starches, proteins, and lipids. Subsequently, Peiris et al. (2010) applied NIR to predict DON levels in wheat kernels, which were found to have low ($<60 \text{ mg kg}^{-1}$) or high ($>60 \text{ mg kg}^{-1}$) DON with an accuracy of approximately 96%. In Southern Brazil, a quantitative analysis of DON and ZEN was conducted through NIR by Tibola et al. (2010); determination coefficient (R^2) values obtained for DON content in wheat kernel and milled wheat were 0.89 and 0.91, respectively, whereas for ZEN content they were 0.86 and 0.87, respectively, thus indicating good prediction results using NIR calibrations. In other studies, Fourier Transform Near-Infrared (FT-NIR) spectrometer was used to analyse DON in durum and common wheat (Girolamo et al. 2009, 2014). The results presented in these papers corroborated the applicability of FT-NIR to analyse large numbers of wheat samples for DON contamination and to confirm the compliance with the European Union (EU) regulation. Dvoracek et al. (2012) also evaluated FT-NIR using 399 winter wheat samples with a known content of DON; the best correlation coefficients were 0.94 ($0 < \text{DON} < 92 \text{ mg kg}^{-1}$) and 0.92 ($0 < \text{DON} < 30 \text{ mg kg}^{-1}$). Nonetheless, classification of wheat flour according to regulated limits of DON via NIR has not yet been scientifically assessed.

Thus, this study aimed at investigating the classification performance of two NIR instruments, dispersive and Fourier transform, in the screening of DON in Brazilian wheat flour samples based on the current MTL of 750 $\mu\text{g kg}^{-1}$ set by ANVISA.

Materials and methods

Wheat flour samples

Two hundred and sixty-seven Brazilian samples of wheat flour (0.3–1.0 kg each) were received, selected and promptly analysed at the Laboratory of Mycotoxicological Analyses (LAMIC), Santa Maria, Brazil, between 2019 and 2020. The material was sent from different mills located in distinct regions of the country. Being part of LAMIC's routine analysis, there was no need for a specific permission to include it in the present assessment; furthermore, it was treated anonymously. The samples did not require grinding because the particle size was $\leq 300 \mu\text{m}$. So, they were homogenised and a fraction was subjected to the process of toxin extraction and subsequently to chromatographic analyses of mycotoxins via liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Another fraction was used for optical data collection in order to build the spectra library. The samples included in this work contained DON levels which were below and above the guidance levels (ANVISA 2011).

Near-infrared analysis

Dispersive infrared spectrometers: spectra were obtained in a Foss XDS Rapid Content® Analyser (FOSS NIRSystems, Hoaganas, Sweden), coupled with a monochromator equipment with a double detector system, silicon (400–1100 nm) and lead sulphide (1100–2500 nm), and wavelength between 400 and 2500 nm, 0.5 nm spectral resolution and 32 spectrum scans. Measurement mode data were acquired in reflectance and subsequently converted to absorbance ($-\log^R$) at the time of modelling. The ring cup was the type of cell used for reading solid samples; each spectrum was read in approximately 1 min. The spectrophotometer was linked to a computer which stored the spectra data collected via the ISIScan software (version 4.8).

Fourier transform Near-Infrared (FT-NIR) spectrometer: spectra acquisition was performed using a FT-NIR spectrometer (MPA, Bruker Optics, Germany) with RT-PbS (lead sulphide) detector and spectral range from 12500 to 3600 cm^{-1} (780–2780 nm), 16 cm^{-1} resolution and 32 sweeps per spectrum. Spectral data were obtained in reflectance,

then converted to absorbance ($-\log^R$) at the time of modelling. The rotating sphere macrosample was the cell type used for reading solid samples; each spectrum was read in approximately 15 s. Reading of samples was performed in duplicate. The spectrophotometer was connected to a computer that stored the data of the spectra collected through the OPUS software (version 6.5).

Deoxynivalenol measurements

A method proposed by Berthiller et al. (2005) was adapted to perform DON analyses. A 3-g sample was added to 24 ml of a methanol: water solution (7:3, v/v) and vortexed for 20 min in a MA563 instrument (Marconi). The extract was then diluted in a methanol:water:ammonium acetate 1 M solution (90:9:1, v/v/v), and 10 μl was injected into a 1200 Series Infinity HPLC (Agilent) coupled to a 4000 QTRAP mass spectrometer (Applied Biosystems), equipped with an ESI source in positive mode. Chromatographic separation was performed with a Zorbax SB-C18 column (4.6 x 150 mm, 5 μm particle diameter) (Agilent) at 40°C. The mobile phase gradient consisted of solutions of methanol:water:ammonium acetate (90:9:1, v/v/v) (solution A) and water: ammonium acetate (90:10, v/v) (solution B). The gradient programme started with 90% A and 10% B for 2 min. Then, phases A and B were kept at 50% for 5 min. At this point, phase A was reduced to 10% and phase B was increased to 90%. During the following minute, phases A and B were maintained at 20% and 80%, respectively. Next, the gradient was increased to reach 100% B for 5 min. In the last 4 min, the gradient programme was returned to the initial conditions of 90% A and 10% B to re-equilibrate the column. Total run time was 17 min. The temperature column was adjusted to 40°C; the injection volume was 50 μL at a flow rate of 1 mL/min.

Method performance parameters

The limit of quantification (LOQ) and the limit of detection (LOD) were established by means of the signal-to-noise ratio (LOQ = 10/1, LOD = 3/1) to ensure analytical quality. Seven spiked replicates were analysed in three different concentration levels for the analyte of interest in order to estimate

recovery of each analyte. Linearity was verified through (R^2), calculated after triplicate injections of analytical curves at seven different concentrations. Analytical curves with $R^2 > 0.99$ were used. The matrix effect for each method was assessed by plotting curves prepared in the solvent vs curves prepared with matrix addition. LOD and LOQ for DON were 50 and 200 $\mu\text{g kg}^{-1}$, respectively.

Spectral treatments and multivariate analysis

The spectral data of the Foss XDS Rapid Content® Analyser was extracted and converted into a JCAMP file, and the MPA data was extracted in opus format; the latter was used in the analyses of multivariate data. The final spectral data were exported to be evaluated with DON data and to conduct the chemometric analyses using the Unscrambler v.9.7 software (CAMO, Norway). Subsequently, spectra from both analysers were pre-processed through the Unscrambler software so that possible spectral variations unrelated to the chemical composition could be removed. To achieve this, the following transformations were performed individually or in a combined way: smoothing; baseline corrections; normalisation (mean, maximum and range); multiplicative scatter correction; and standard normal variate. The equations which showed the best calibration and validation results were chosen.

Development of calibration models

Partial Least Squares Discriminant Analysis (PLS-DA) and Principal Component Analysis-Linear Discriminant Analysis (PC-LDA) were applied to classify the wheat flour samples based on the levels of DON. Firstly, 450 $\mu\text{g kg}^{-1}$ was taken as a fixed value of DON; this level considered the current legislation (750 $\mu\text{g kg}^{-1}$) and the uncertainty of the reference methodology ($\pm 200 \mu\text{g kg}^{-1}$). Next, the samples were classified according to the permitted level (750 $\mu\text{g kg}^{-1}$), with two groups being established for the calibration set: category A ($\leq 450 \mu\text{g kg}^{-1}$), non-contaminated or below the MTL; and category B ($> 450 \mu\text{g kg}^{-1}$), contaminated or above the MTL. Thus, 227 samples constituted the calibration database, and 40 samples were used for validation. The validation set was built with

samples that were not in the calibration set, i.e. unknown samples. For that, two groups were formed following the same parameters of the calibration: category A ($\leq 450 \mu\text{g kg}^{-1}$), non-contaminated; and category B ($> 450 \mu\text{g kg}^{-1}$), contaminated.

Discriminant analysis is a supervised and qualitative classification method used to create classification rules with several predefined classes. After defining the classes, the models are used to assign unknown samples to their most probable class. Unlike regression, which predicts the values quantitatively, the discriminant analysis classifies the population through a categorical variable that may be interpreted as classes to which a sample may belong. PLS-DA is a classification method based on modelling the differences between two or more classes using the PLS regression method. The construction method of the data matrix used to discriminate the classes was +1 for members of class A and -1 for members of class B; the fictitious variable for groups was attributed to 1, if the sample belonged to the group, or zero, if the sample did not belong to the group. The PC-LDA is a classificatory technique which combines exploratory analysis of the data aiming at dimensionality reduction, with no class discrimination, using Principal Components (PC) with Linear Discriminant Analysis (LDA), which considers the existence of classes for the data. The following two groups were the levels of categorical variable used to develop the data matrix: low ($\leq 450 \mu\text{g kg}^{-1}$), non-contaminated, and high ($> 450 \mu\text{g kg}^{-1}$), contaminated. Linear and quadratic models and Mahalanobis distance may be used as LDA classifiers. The final evaluation of the models is performed through a confusion matrix.

Performance of the classification models was evaluated according to the calibration that showed the highest accuracy and the lowest false positive (FP) and false negative (FN) indices. FP is the percentage of samples incorrectly classified as belonging to group A divided by the total number of samples in this group, while FN is the percentage of samples incorrectly classified as belonging to group B divided by the total number of samples within this group. The accuracy percentage of the model was calculated considering the number of samples from Groups A and B, correctly broken

down by the total number of samples in the calibration set (overall discrimination rate, ODR).

Results

Descriptive data statistics

The spectra originated from dispersive NIR and FT-NIR instruments with wheat flour samples including low ($<200 \mu\text{g kg}^{-1}$), intermediate ($467 \mu\text{g kg}^{-1}$) and high concentrations ($>1,201 \mu\text{g kg}^{-1}$) are presented in Figure 1. DON levels varied from 200 to $1,690 \mu\text{g kg}^{-1}$ (range of $1,490 \mu\text{g kg}^{-1}$); mean value, standard deviation and median were 497, 358, and $361 \mu\text{g kg}^{-1}$, respectively. Histograms of DON concentrations based on the results of LC-MS/MS are shown in Figure 2. In the calibration dataset, 58% of the samples ($n = 132$) presented concentrations lower than or equal to $450 \mu\text{g kg}^{-1}$, while 42% ($n = 95$) were above this level. Despite the smaller

proportion of greater concentrations, the levels were representative for building the calibrations.

Classificatory methods

Forty samples of wheat flour with varying levels of DON contamination were used to evaluate and validate the classification models (PLS-DA and PC-LDA). DON levels varied from 200 to $1,320 \mu\text{g kg}^{-1}$ (range of $1,120 \mu\text{g kg}^{-1}$); mean value, standard deviation, and median were 518, 301, and $457 \mu\text{g kg}^{-1}$, respectively.

PLS-DA

The validation results obtained using PLS-DA through different spectral pre-treatments and their combinations are displayed in Table 1.

The best mathematical treatments found for dispersive NIR and FT-NIR were respectively Savitzky–Golay smoothing combined with normalisation

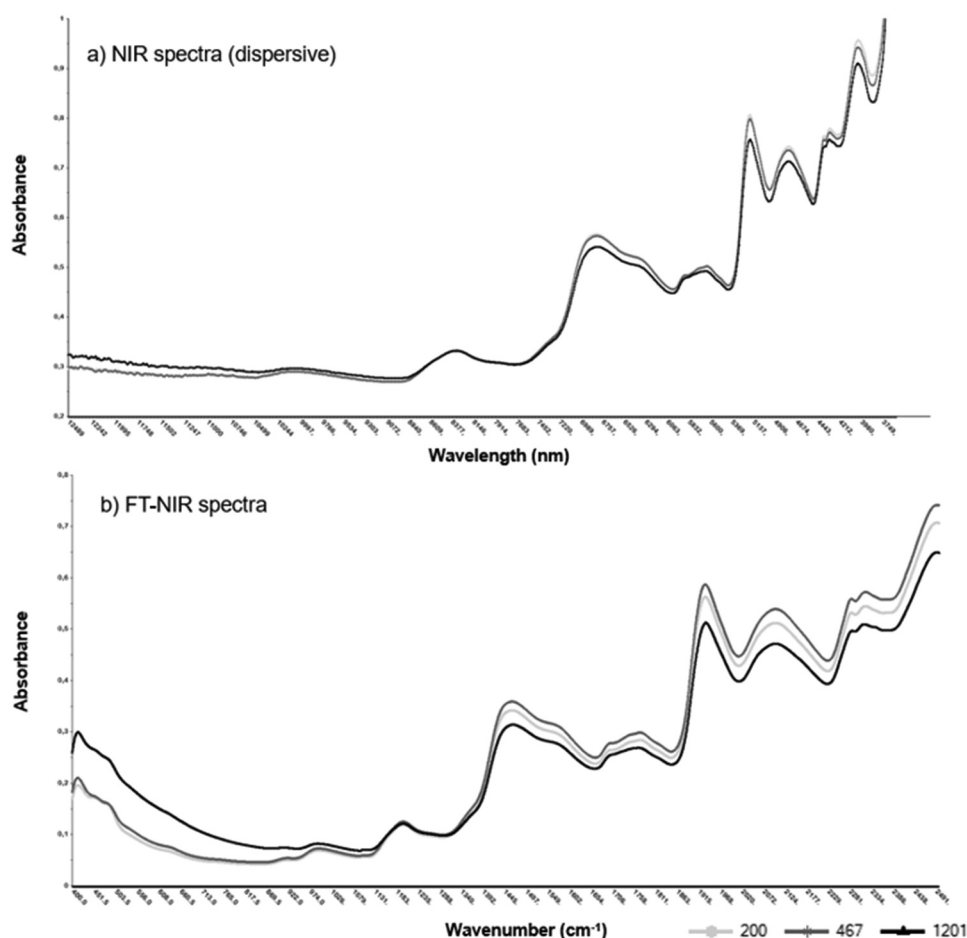


Figure 1. Raw spectra of wheat flour samples read in two instruments: (a) dispersive Near-Infrared spectroscopy (NIR), and b) Fourier transform Near-Infrared (FT-NIR). Three different concentrations of DON were included.

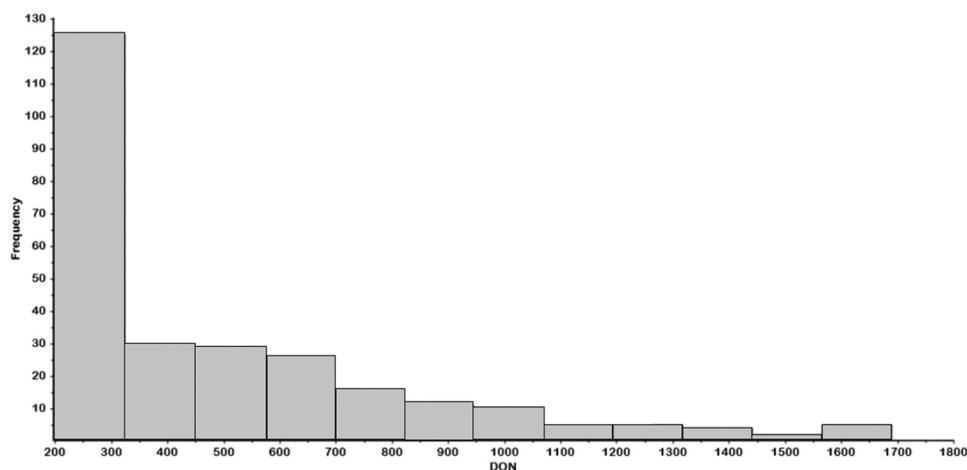


Figure 2. Frequency and concentration range of DON in wheat flour samples ($n = 227$) used in the calibration set.

Table 1. Results of the different mathematical pre-treatments in the dispersive Near-Infrared Spectroscopy (NIR) and Fourier transform Near-Infrared (FT-NIR) instruments obtained via Partial Least Squares Discriminant Analysis (PLS-DA) .

Pre-treatment	Classification method (PLS-DA)					
	NIR (dispersive)			FT-NIR		
	ODR (%)	FP (%)	FN (%)	ODR (%)	FP (%)	FN (%)
Raw spectra	77.5	15	30	80.0	15	25
Smoothing (moving average)	82.5	15	20	77.5	10	35
Smoothing (Savitzky–Golay – 2,4,4)	80.0	10	30	87.5	10	15
Smoothing (Savitzky–Golay –1,4,4)	80.0	10	30	80.0	20	20
SNV-Baseline-MSC	80.0	15	25	72.5	20	35
Smoothing-detrending	75.0	25	25	75.0	25	25
Smoothing-normalisation (mean)	80.0	15	25	77.5	20	25
Smoothing-normalisation (maximum)	85.0	15	15	80.0	15	25
Smoothing-normalisation (range)	82.5	10	25	77.5	10	35

ODR: overall discrimination rate; FP: false positive; FN: false negative; Smoothing (Savitzky–Golay – 2,4,4): Savitzky–Golay smoothing (second order polynomial; gap segment left: 4; gap segment right: 4); Smoothing (Savitzky–Golay –1,4,4): Savitzky–Golay smoothing (first order polynomial; gap segment left: 4; gap segment right: 4); SNV: standard normal variate; MSC: multiplicative scatter correction. Cut-off point set at 450 $\mu\text{g kg}^{-1}$.

(maximum), and Savitzky–Golay smoothing. The ODR in the dispersive NIR was 85%; both FP and FN rates were 15% (Figure 3). The ODR in the FT-NIR was 87.5%; the FP and FN rates were 10% and 15%, respectively (Figure 4).

PC-LDA

The validation results obtained using PC-LDA through different spectral pre-treatments and their combinations are shown in Table 2. The best mathematical treatments found for dispersive NIR and FT-NIR were respectively Savitzky–Golay smoothing combined with normalisation (maximum), and Savitzky–Golay smoothing combined with normalisation (range). The ODR in the dispersive NIR was 90%; both FP and FN rates were 10%. The ODR in the FT-NIR was 87.5%; the FP and FN indices were 10% and 15%, respectively.

Discussion

Global food supply is a key issue from the nutritional and health perspectives. Wheat is one of the primary sources of nutrients for the population, considering its worldwide consumption and use. Nonetheless, this major cereal grain faces a serious threat from fungal attack and the consequent development of mycotoxins, especially DON. The most efficient way to avoid human intoxication by such toxins is to prevent fungal development by applying good agricultural practices or selecting genetically modified cultivars which are more resistant to the attack of toxigenic fungi (Lee and Ryu 2017). However, climatic variations play an important role in fungal development and occurrence (Prandini et al. 2009).

In southern Brazil, the highest incidence of DON in cereals occurs in winter, when temperature and

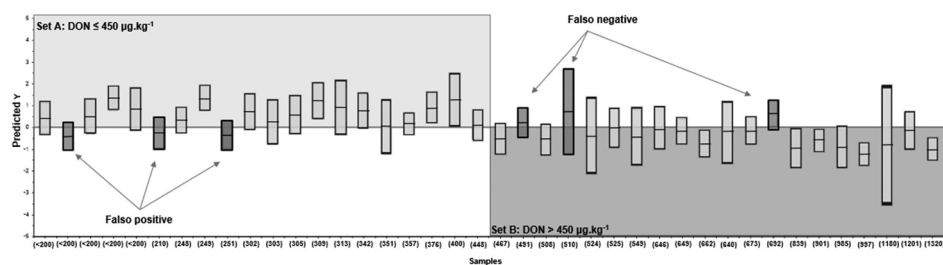


Figure 3. Results of the validation samples obtained through PLS-DA using dispersive Near-Infrared Spectroscopy (NIR) for DON analyses. The red samples were considered as FP when belonging to set A ($\text{DON} \leq 450 \mu\text{g kg}^{-1}$) and FN when included in set B ($\text{DON} > 450 \mu\text{g kg}^{-1}$).

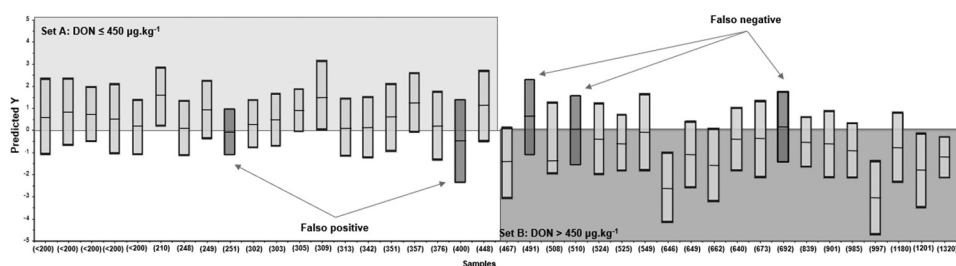


Figure 4. Results of the validation samples obtained through PLS-DA using Fourier Transform Near-Infrared (FT-NIR) for DON analyses. The red samples were considered as FP when belonging to set A ($\text{DON} \leq 450 \mu\text{g kg}^{-1}$) and FN when included in set B ($\text{DON} > 450 \mu\text{g kg}^{-1}$).

Table 2. Results of the different mathematical pre-treatments in the dispersive Near-Infrared Spectroscopy (NIR) and Fourier transform Near-Infrared (FT-NIR) instruments obtained via Principal Component Analysis-Linear Discriminant Analysis (PC-LDA).

Pre-treatment	Classification method (PC-LDA)					
	NIR (dispersive)			FT-NIR		
	ODR (%)	FP (%)	FN (%)	ODR (%)	FP (%)	FN (%)
Raw spectra	80.0	25	15	80.0	15	25
Smoothing (moving average)	83.0	20	15	80.0	15	25
Smoothing (Savitzky-Golay – 2,4,4)	85.0	20	10	77.5	15	30
Smoothing (Savitzky-Golay – 1,4,4)	85.0	20	10	85.0	10	20
SNV-Baseline-MSC	80.0	30	10	87.5	0	25
Smoothing-Detrending	75.0	10	40	85.0	10	20
Smoothing-normalisation (mean)	85.0	20	20	80.0	15	25
Smoothing-normalisation (maximum)	85.0	10	20	87.5	10	15
Smoothing-normalisation (range)	90.0	10	10	80.0	15	25

ODR: overall discrimination rate; FP: false positive; FN: false negative; Smoothing (Savitzky-Golay – 2,4,4): Savitzky-Golay smoothing (second order polynomial; gap segment left: 4; gap segment right: 4); Smoothing (Savitzky-Golay – 1,4,4): Savitzky-Golay smoothing (first order polynomial; gap segment left: 4; gap segment right: 4); SNV: standard normal variate; MSC: multiplicative scatter correction. Cut-off point set at $450 \mu\text{g kg}^{-1}$.

humidity favour fungal growth (Del Ponte et al. 2012). DON prevalence in Brazilian wheat has been reported with significant rates. In recent years, positivity has ranged from 65% to 73%, with averages between 660 and 1894.9 $\mu\text{g kg}^{-1}$ (Sifuentes dos Santos et al. 2013; Machado et al. 2017; Mallmann et al. 2017). DON can be present even after wheat grain processing; therefore, it may be found in by-products such as flour. Nevertheless,

DON contamination levels are reduced in wheat flour compared to wheat and wheat bran due to the cleaning and sorting procedures used in foods destined for human consumption (Tibola et al. 2015). Even so, contamination can occur at levels that exceed the legislation limits. Lanza et al. (2018) reported 100% prevalence of DON in wheat flour samples ($n = 39$), with concentrations ranging from 158 to 1,606 $\mu\text{g kg}^{-1}$; furthermore, 23% of the

contaminated samples presented levels above the MTL. The Brazilian current legislation establishes the MTL for DON in wheat flour at $750 \mu\text{g kg}^{-1}$ (ANVISA 2011).

Optical methods have become increasingly important as quick methodologies for the qualification and/or quantification of substances. These techniques meet the new environmental demands for presenting benefits, including real-time and non-destructive analysis of samples; minimal sample preparation; simultaneous prediction of different parameters based on a single spectrum; non-invasive character; and no waste generation (Lim et al. 2018).

This study investigates the use and applicability of the near-infrared technology in Brazilian wheat flour samples with different levels of DON through two classification methods: PLS-DA and PC-LDA. Dispersive NIR and FT-NIR were used to find the predictions which best discriminate samples in category A ($\text{DON} \leq 450 \mu\text{g kg}^{-1}$) from samples in category B ($>450 \mu\text{g kg}^{-1}$).

Some mycotoxicological evaluations have employed dispersive equipment and FT-NIR to analyse an assortment of cereals; these studies mainly assess DON contamination in wheat and wheat bran via quantification and qualification methods, but not in wheat flour (Girolamo et al. 2009, 2014, 2019; Peiris et al. 2016). Thus, evaluation of DON in Brazilian wheat flour samples by using NIR is reported here for the first time. Figure 1 shows the raw spectra of the samples representing varying levels of contamination. It is not feasible visually to distinguish (with and without contamination) and to analyse the spectra without making use of a multivariate analysis tool. The entire range ($400\text{--}2500 \text{ nm}$ and $3600\text{--}12500 \text{ cm}^{-1}$) was used for the evaluation of the classification models, since the spectral region in which DON is present remains to be determined. The measurement is based on changes caused by fungal contamination and, consequently, alterations in the chemical structure of the grain. *Fusarium* species with mycotoxigenic potential are known to cause changes in carbohydrate and protein content, leading to the development of mycotoxins (Balut et al. 2013).

Table 1 displays the findings achieved with dispersive NIR and FT-NIR via PLS-DA. Both instruments proved to be efficient in distinguishing

samples in category A ($\text{DON} \leq 450 \mu\text{g kg}^{-1}$) from samples in category B ($>450 \mu\text{g kg}^{-1}$). Distinct pre-treatments were used aiming to determine the best discrimination models. This allowed obtaining an ODR over 80% (85% and 87.5% with dispersive NIR and FT-NIR, respectively). A total of six (FP and FN rates of 15%) and five (FP rate of 10% and FN rate of 15%) samples were incorrectly predicted when using dispersive NIR (Figure 3) and FT-NIR (Figure 4), respectively. The present findings are in line with those reported by Girolamo et al. (2019) when analysing DON-contaminated wheat bran samples via PLS-DA; the authors found an ODR of 87%.

When the percentages of FP and FN are evaluated, the latter represent the most worrisome scenario, since an inadequate product may be used and consumed. A deeper analysis of the FN indices obtained with both instruments indicates that two samples presenting DON at 491 and $510 \mu\text{g kg}^{-1}$ were considered as FN (group A) according to the criteria established by the groups. However, considering the uncertainty of the reference methodology (LC-MS/MS), which is $\pm 200 \mu\text{g kg}^{-1}$, the concentrations of such samples would be 491 ± 200 and $510 \pm 200 \mu\text{g kg}^{-1}$; thus, DON levels would be below the MTL of $750 \mu\text{g kg}^{-1}$. Therefore, relative to the $\mu\text{g kg}^{-1}$ regulatory limit, the FN rates are reduced to 5% and 10%, and the ODR increases to 90% and 92.5% in NIR and FT-NIR, respectively.

Table 2 presents the results obtained with dispersive NIR and FT-NIR via PC-LDA. As seen for PLS-DA, both instruments had an excellent performance and showed an ODR above 80%. Both FP and FN rates were 10% with dispersive NIR; when FT-NIR was used, FP and FN indices were 10% and 15%, respectively. Levasseur-Garcia and Kleiber (2015) evaluated the discriminant analysis method in maize kernels which were naturally contaminated by DON and fumonisins; accuracy varied from 60% to 84%, depending on the model applied. In another study, Girolamo et al. (2014) investigated the classification models to predict DON levels in durum wheat samples and found an ODR varying between 75% and 90%; false compliant rates ranged from 3% to 7%.

Both dispersive and FT-NIR instruments showed similar classification performance, with no advantage of one methodology over the other. The same was true for the evaluated discriminatory methods,

since good discrimination rates and low FP and FN indices were found for both models. NIR is a leading technology due to its ease of use; a variety of instruments is available in the market, and the difference between them lies in the way the spectral collection takes place and in the intended use. The instruments tested here can read the sample in less than a minute and are considered environmentally friendly, for they do not produce chemical residues.

PLS-DA and PC-LDA showed an ODR above 80% for both dispersive NIR and FT-NIR. Thus, they represent alternative optical tools to promptly analyse wheat flour in the industry. It is worth mentioning that the samples which were found to belong to category B ($>450 \mu\text{g kg}^{-1}$) should not be discarded; instead, they should be reanalysed through the reference methodology in order to confirm the results, which is commonly done in routine analysis. Therefore, this work presents alternatives to evaluate a greater number of lots compared to the traditional techniques most typically employed in the industry, and thus assist in the decision-making process.

Despite the promising outcomes of this study, calibrations are not static. New samples representing the reality in the field should be included in the database along the next years in order to improve robustness of the technique.

Conclusion

The results reported here confirm that dispersive NIR and FT-NIR may be used as alternative methods to classify wheat flour according to DON content. Both classification methods applied in the study, PLS-DA and PC-LDA, achieved accuracy rates over 80%, thus demonstrating a high discrimination rate. Such findings indicate that NIR may be used as a screening method to evaluate DON contamination in wheat flour, considering the limits set by the current Brazilian legislation. Furthermore, this rapid and easy-to-perform tool can enable analysis of various lots, thus supporting decision making in the Industries.

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Disclosure statement

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