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Research Paper

Deoxynivalenol screening in wheat kernels using hyperspectral imaging

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The use of hyperspectral imaging (HSI) for deoxynivalenol (DON) screening in wheat kernels is investigated. Experiments were carried out using a new algorithm designed to be simple to implement and computationally light, being largely based on the manipulation of a few selected spectral bands. Initial experimental results revealed that direct estimation of DON content using hyperspectral images is currently unfeasible, but they also indicated that an indirect analysis exploring the correlation between Fusarium damage and DON content may be accurate enough to improve the process of DON screening in the production chain. This motivated the adoption of a classification approach, in which an algorithm, instead of estimating a value for the DON content, classifies wheat kernel batches into two or three categories, depending on the application. The developed algorithm achieved accuracies of 72% and 81% for the three- and two-class classification schemes, respectively. The results, although not accurate enough to provide conclusive screening, indicated that the algorithm could be used for initial screening to detect wheat batches that warrant further analysis regarding their DON content.

1. Introduction

Mycotoxins are secondary metabolites produced naturally by filamentous fungi, which are considered as hazardous when present in food for humans and feed for animals (Rocha, Freire, Maia, Guedes, & Rondina, 2014). *Fusarium graminearum sensu stricto* (Fgss) is the predominant species in Brazil, and is a potential deoxynivalenol (DON) producer, according to molecular methods that characterise genes involved in the synthesis of trichothecenes (Del Ponte et al., 2015).

DON poses a significant threat to both humans and animals, as it can interfere with normal eukaryotic cell function by inhibiting protein synthesis (Rotter, 1996; Schmale and Bergstrom, 2003). Many countries have already established limits for DON levels. In the European Union (EU), the limits for DON in wheat are 0.2 mg kg^{-1} for baby food, 0.5 mg kg^{-1} for bread and pastries, 0.75 mg kg^{-1} for pasta and 1.25 mg kg^{-1} for unprocessed cereals (Cheli, Battaglia, Gallo, & Dell'Orto, 2014; EFSA, 2013). In Brazil, the upper limits established for DON for whole wheat and white flour are 2.00 mg kg^{-1} and 1.75 mg kg^{-1}, respectively (ANVISA, 2011). Thus, to avoid health risks, DON levels must be estimated before the cereals are processed and incorporated to human and animal diets.

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Nomenclature

DC-ELISA	Direct competitive enzyme-linked immunosorbent assay
DON	Deoxynivalenol
DPI	DON Preliminary Index
EU	European Union
FDK	Fusarium-Damaged Kernels
Fgss	Fusarium graminearum sensu stricto
FHB	Fusarium Head Blight
FPA	Focal Plane Array
FT-NIR	Fourier Transform Near-Infrared
HSI	Hyperspectral Imaging
LC–MS	Liquid chromatography–mass spectrometry (LC–MS)
NIR	Near-Infrared
PDF	Probability density function
ppm	Parts per million
RI	Reference Image
ROI	Region of interest
VIS	Visible Range

Currently, there are several methods for estimating DON content. The most common ones are chromatography and immunochemical techniques. Since they provide relatively accurate estimates, their measurements are usually used in the wheat commerce. However, they are regarded as too time consuming for a rapid screening, and they often require laboratory facilities (Schaafsma, Savard, Clear, & Dexter, 2004). Descriptions of some commonly used chemical methods can be found in Miedaner et al. (2004) and Meneely, Ricci, van Egmond, and Elliott (2011).

Another commonly used approach is using visual estimates for the Fusarium-Damaged Kernels (FDK) to indicate the presence of DON. Fusarium causes kernels to shrivel and to become whitish, reducing protein content (Jin et al., 2014). Delwiche, Kim, and Dong (2011) found that the spectral bands that are more affected by Fusarium are 1200 nm (due to the presence of ergosterol) and 1420 nm (probably due to reduced water content). Since the FDK estimation is a subjective task, it is subject to well-known psychological and cognitive distortions (Bock, Poole, Parker, & Gottwald, 2010). The possibility of using FDK to estimate DON levels has been investigated by Paul, Lipps, and Madden (2005). They conducted a study in which four types of visual analyses were used to estimate DON levels, with FDK achieving the best correlation (0.73). Recently, some methods for estimating FDK (or some equivalent measurement) automatically have been proposed (Barbedo, Tibola, & Fernandes, 2015; Maloney et al., 2014). However, despite their relatively good results, the errors inherent in these tools cascade with the error of using FDK to estimate DON levels, reducing their reliability.

Liu, Elen, Sundheim, Langseth, and Skinnes (1997) investigated the freezing blotter test to infer DON levels. Although the correlations were slightly better than those obtained using FDK, the classification of the kernels as heavily, medium and lightly infested depends on careful microscopic analysis, which slows the process.

The use of Near-Infrared (NIR) reflectance spectroscopy in plant sciences has increased steeply. The main problem with this technique is that the DON content estimation is based on factors such as kernel hardness and colour, which are only indirectly linked with the presence of DON (Schaafsma et al., 2004). Also, until now, reliable results were only obtained for high DON concentrations, much above those considered safe. Dowell, Ram, and Seitz (1999) used this approach in the 400–1700 nm band, succeeding in detecting individual wheat kernels with DON levels above 120 mg kg^{-1}. Peiris et al. (2010) and Jin et al. (2014), using the Single Kernel Near Infrared (SKNIR) system, also successfully detected kernels with high DON concentrations. Pettersson and Aberg (2003), using the 670–1100 nm band, were successful for cases slightly above EU regulation levels. Beyer, Pogoda, Ronellenfitsch, Hoffmann, and Udelhoven (2010) proposed to estimate DON levels using the 350–2500 nm wavelength region; however, the standard error observed included the EU limits, making their method too imprecise to reliably distinguish grain batches with DON levels above these limits.

One variation of the NIR spectroscopy is the Fourier Transform NIR (FT-NIR). This technology is based on interferometry, as opposed to the diffraction-based ones, producing better resolution and throughput (Sivakesava & Irudayaraj, 2000), with a comparatively lower photometric response. This was the approach adopted by Girolamo, Lippolis, Nordkvist, and Visconti (2009), which achieved an accuracy of 69% in the identification of kernels with DON levels above 0.3 mg kg^{-1}. Dvořáček, Prohasková, Chrpová, and Štočková (2012) also adopted this strategy, which gave errors above EU limits but which were low enough for application in disease resistance experiments. Girolamo, Cervellieri, Visconti, and Pascale (2014) applied FT-NIR for classifying durum wheat kernels into three classes according to their DON levels, achieving an accuracy of 75%.

Hyperspectral imaging (HSI) is the most recent technique to be explored for analysing wheat kernels. This technique uses the same principles of spectroscopy, but it generates spectra for each pixel in an image, rather than for a small localized area. Delwiche, Souza, and Kim (2013) tested this approach for estimating three quality properties for wheat kernels, concluding that HSI still cannot replace actual pilot milling procedures. Barbedo et al. (2015) used this technique for detecting Fusarium head blight in wheat kernels. They concluded that hyperspectral imagery is currently not sensitive enough to estimate DON content directly, but than an indirect estimation from Fusarium damage could be achieved with relative success.

To the best of our knowledge, the algorithm described in this paper is the first aimed at detecting DON in wheat kernels using hyperspectral images. The motivation for using this kind of technique is its potential to be a core component of rapid screening systems for DON content, which may soon become a fast and cost-effective alternative to chemical analysis and visual inspection.

The proposed method used seven spectral bands (from the 256 originally contained in the hyperspectral images used in its calibration) for indirect detection of DON in wheat kernels. Five of those bands were used for segmenting the kernels from the background, and the remaining two were used for