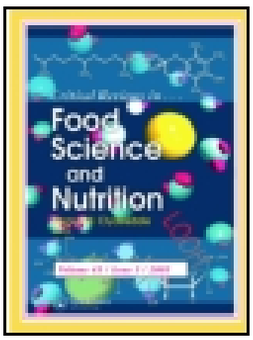

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Recent developments and applications of hyperspectral imaging for rapid detection of mycotoxins and mycotoxigenic fungi in food products

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ABSTRACT

Mycotoxins are the foremost naturally occurring contaminants of food products such as corn, peanuts, tree nuts, and wheat. As the secondary metabolites, mycotoxins are mainly synthesized by many species of the genera *Aspergillus, Fusarium* and *Penicillium*, and are considered highly toxic and carcinogenic to humans and animals. Most mycotoxins are detected and quantified by analytical chemistry-based methods. While mycotoxigenic fungi are usually identified and quantified by biological methods. However, these methods are time-consuming, laborious, costly, and inconsistent because of the variability of the grain-sampling process. It is desirable to develop rapid, non-destructive and efficient methods that objectively measure and evaluate mycotoxins and mycotoxigenic fungi in food. In recent years, some spectroscopy-based technologies such as hyperspectral imaging (HSI), Raman spectroscopy, and Fourier transform infrared spectroscopy have been extensively investigated for their potential use as tools for the detection, classification, and sorting of mycotoxins and toxigenic fungal contaminants in food. HSI integrates both spatial and spectral information for every pixel in an image, making it suitable for rapid detection of large quantities of samples and more heterogeneous samples and for in-line sorting in the food industry. In order to track the latest research developments in HSI, this paper gives a brief overview of the theories and fundamentals behind the technology and discusses its applications in the field of rapid detection and sorting of mycotoxins and toxigenic fungi in food products. Additionally, advantages and disadvantages of HSI are compared, and its potential use in commercial applications is reported.

KEYWORDS

Non-destructive methods; hyperspectral imaging; mycotoxins; mycotoxigenic fungi; classification

Introduction

Mycotoxins are low-molecular-weight natural products produced as secondary metabolites by filamentous fungi (Bennett and Klinch, 2003). These metabolites constitute a toxigenically and chemically heterogeneous assemblage that are grouped together only because the members can cause disease and death in human beings and other vertebrates (Bennett 2010; Berthiller et al. 2013; Rodriguez et al., 2012). Mycotoxins are mainly synthesized by many species of the genera *Aspergillus, Fusarium* and *Penicillium* (Terra et al., 2012). The mycotoxins, aflatoxins (AFTs) have been classified by the International Agency for Research on Cancer as group 1 carcinogens, ochratoxin A and fumonisins as group 2B, and deoxynivalenol and zearalenone as group 3 carcinogens. Mycotoxins and toxigenic fungi contamination of foods and feeds are concerned not only by consumers, retailers and regulatory authorities due to their safety risks, but also by producers because of their potential economic impact (He and Sun, 2015). As a consequence, more than 100 countries and organizations have established strict regulations for managing risks posed by mycotoxins in food and feed (Moss, 2008). Moreover, regulatory pressure is increasingly important with the implementation of specific norms for the control of mycotoxins (Campagnoli et al., 2011; Fredlund et al., 2009). Therefore, identification, inspection, classification and control of mycotoxins contamination are particularly important for the safety of foods. The ability to rapidly detect toxigenic fungi was maybe helpful to predict and control the risk of mycotoxins contamination in food (Lecellier et al., 2015).

Over the years, effective methods for detecting mycotoxins and toxigenic fungi in food products have been developed and reported. The current approved methods for mycotoxin detection are expensive chemical-based analytical methods such as high performance liquid chromatography (Selvaraj et al., 2015) and liquid chromatography-mass spectrometry (Ediage et al. 2012). The steps involved in the food inspection process include initial sampling, mixing of samples, sub-sampling, sample grinding, sub-sampling of ground materials, and final chemical analysis (USDA, 2002). Consequently, the detection process is time-consuming, laborious and expensive. Furthermore, organic reagents such as methanol, acetonitrile, *n*-hexane and ethyl acetate are always necessary, and most of them are poisonous and dangerous to humans (He and Sun, 2015). At present, there are several rapid screening methods,

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such as the enzyme-linked immunosorbent assay, lateral flow immunoassay devices (cards and strips), fluorometric assays with immunoaffinity column cleanup, and recent biosensors (Selvaraj et al., 2015; Zheng, Richard and Binder, 2006). However, these rapid methods still require minimal sample preparation, destruction, mixing and extraction of samples.

Adequate sampling is a significant part of the accurate determination of mycotoxins using the above methods. In these approaches, the mycotoxin concentration of a bulk lot is often estimated by measuring the concentration in a small portion of the lot or in a sample taken from the lot (Whitaker, 2006). However, previous studies have illustrated that only a few individual kernels or seeds in any grain lot may be contaminated with the mycotoxin, with very high concentrations associated with these kernels (Campbell et al., 1986). Thus, capturing these contaminated kernels is a challenge in sampling. Sample size has a direct impact on the accuracy of the analysis result (Whitaker et al., 1993). As a consequence, sampling is often the largest source of variability for small sample sizes, and it contributes to the measurement uncertainty about the true value for mycotoxins in food item lots (Whitaker, 2003). The lack of adapted tools for the food and feed sectors may lead to inaccurate evaluation of the extent of mycotoxin contamination and uncontrolled risk for these industries. Therefore, it is desirable to develop rapid, effective and efficient non-destructive methods for the detection of mycotoxins in food. Using such methods to screen a large quantity of grains can greatly reduce the sampling error, thereby increasing the sample size for analysis.

A typical method for analyzing the load of toxigenic fungi is the plate counting method, which is done by plating dilutions on a single culture plate and then recording colonies within 30–300 (ISO, 2003). Here, the result is always expressed as an order of magnitude such as 1×10^n CFU/g. Furthermore, routine identification of fungi mainly relies on phenotypic methods, which often require expertise in morphological analysis (Marinach-Patrice et al., 2009). In recent years, DNA-based methods such as polymerase chain reaction and sequencing of the internal transcribed spacer region or other special genes have been developed and made available. Although the results these methods provide are objective and reliable, they are time-consuming, laborious, costly, destructive and difficult to implement in routine laboratory practices (Nilsson et al., 2009).

In recent years, research interest in spectroscopy techniques has been thriving because of their potential application in rapid and non-destructive analysis for food quality and safety and because of their minimal or obviated sample preparation. Raman spectroscopy, Fourier transform infrared (FTIR) spectroscopy and hyperspectral imaging (HSI) are the main spectroscopic methods for detecting mycotoxins and toxigenic fungi in food products (Alexandrakis et al., 2012; Lu et al., 2011; Yoshimura et al., 2014). Although several studies have reported positive results for the rapid detection or classification of mycotoxins and toxigenic fungi in food using Raman spectroscopy and FTIR spectroscopy, both methods are destructive and only give average values of a small section of a small homogeneous sample. Therefore, they are not suitable for rapid, non-destructive analysis of large quantities of samples and more heterogenous samples. Moreover, both offer only constituent gradients and are unable to provide information on the spatial distribution in samples (Liu, Zeng and Sun, 2015). The HSI method, which integrates both imaging and spectral information for every pixel in an image, is suitable for large quantities of samples (Barbin et al., 2012; Costa et al., 2011; ElMasry et al., 2012a; ElMasry et al., 2012b; Jackman et al., 2009; Kamruzzaman et al., 2012; Sun, 2004; Wang and Sun, 2002; Wu, Sun and He, 2012; Wu and Sun, 2013). Therefore, this paper is aimed at reviewing the potential use of HSI in detecting mycotoxins and toxigenic fungi in food products.

Hsi technique

Different from traditional spectroscopy, which provides spectral information only, HSI can provide both spectral information and spatial images through the use of traditional computer vision technology (Costa et al., 2011; He and Sun, 2015; Jackman et al., 2009; Sun, 2004). This characteristic enables the use of HIS in characterizing the target food more objectively and reliably (Barbin et al., 2012a; Barbin et al., 2012b; ElMasry et al., 2012a; Kamruzzaman, et al., 2012; Wu et al., 2012; Wu and Sun, 2013). Depending on the applications, a HSI system can cover a specific spectral range in the ultraviolet (UV), visible near-infrared (VNIR), or shortwave infrared (SWIR) region. A typical HSI system mainly consists of a spectrograph, a camera with lens, an illumination unit, a moving component driven by a motor, and a computer with installed image acquisition software (Figure 1). By scanning with such a system, a hyperspectral image of a food product can be generated. It can then be used as a basis for further product safety and quality evaluation (Sun, 2010). Recent progresses in and applications of HSI for detecting various mycotoxins and mycotoxigenic fungi are reviewed in the following sections.

Applications of HSI for classification of aflatoxins contaminated food products

Aflatoxin, a secondary metabolite of *A. flavus* and *A. parasiticus*, is the most potent naturally occurring toxic and hepatocarcinogenic compounds (Wu, 2014). Aflatoxin contamination of maize is a serious and long-standing problem. Detection, classification and sorting prior to harvest, storage and processing may aid the removal of the infected grains, which can also be done with rapid and non-destructive techniques. Several studies have reported positive results for rapid classification of aflatoxin-contaminated food products by using HSI (Table 1). Yao et al. (2010) used a fluorescence VNIR HSI system in the 400–600 nm to determine aflatoxin levels in single artificially contaminated maize kernels. A general negative correlation was found between the measured aflatoxin concentration and the fluorescence image bands in the blue and green regions. The correlation coefficient (r^2) was 0.72 for the multiple linear regression (MLR) models obtained by the multivariate analysis of variance (MAV). When a threshold of either 20 or 100 μg/kg was set, the classification accuracy under a two-class schema was found to range within 0.84 to 0.91, which indicates that fluorescence HSI is applicable to predicting the aflatoxin content of individual maize kernels. Yao et al. (2013a) applied a fluorescence HSI system in the 400–700 nm region to assess the difference in fluorescence emissions between maize kernels

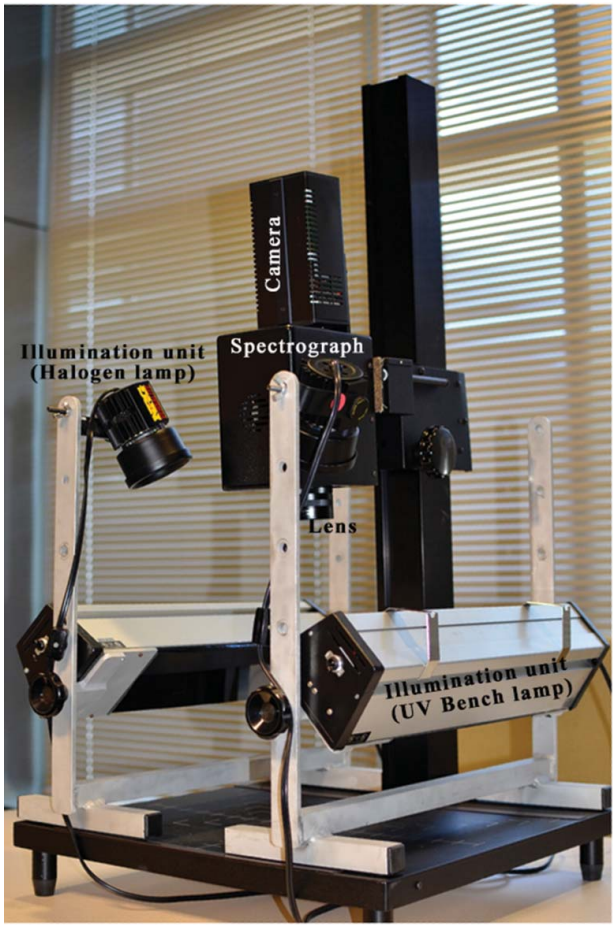

Figure 1. Illustration of the fluorescence hyperspectral imaging system (Yao et al., 2008).

field-inoculated with toxigenic and atoxigenic *A. flavus*. The contaminated maize kernels all had peak wavelengths longer than those of the healthy samples. For the endosperm side and germplasm side, the peak fluorescence differences were 12 nm and 9 nm, respectively. A classification accuracy (94.4%) was achieved for the 100 μg/kg threshold on the germ side, which is higher than that for the 20 μg/kg threshold. Yao et al. (2013b) used a fluorescence HSI system in the 400–900 nm to classify single corn kernels contaminated with aflatoxin by field inoculation. Using binary encoding analysis, they achieved slightly higher classification accuracies of 87% and 88%, when they used 20 μg/kg and 100 μg/kg, respectively, as the classification thresholds. The correlation was −0.81 with a normalized difference fluorescence index when they used the bands at 437 nm and 537 nm. Furthermore, Hruska et al. (2013) used a visible and NIR HSI system in the 400−900 nm range under UV excitation to assess the spectral differences between field-inoculated maize kernels with two aflatoxigenic *A. flavus* strains and naturally infected maize from the same field. Spectral analysis based on contaminated "hot" pixel classification showed a significant spectral shift between contaminated and healthy corn ears with fluorescence peaks at 501 and 478 nm, respectively. All inoculated and naturally infected control ears had fluorescence peaks at 501 nm, which are different from those of the uninfected samples. In order to improve the prediction accuracy of detection and classification, Zhu et al. (2016) investigated the integration of fluorescence and reflectance VNIR hyperspectral images to detect aflatoxin contamination in whole field-inoculated corn kernels. By using a least squares support vector machines (LS-SVM) model with the 20 μg/kg and 100 μg/kg thresholds, the overall classification accuracy was 90.00%-93.33%, 92.67%-94.00% and 90.00%-95.33% for fluorescence, reflectance and integrated spectra, respectively. The germ side was confirmed to be better than the endosperm side, especially in terms of the true positive rate (TPR). The integrated analysis achieved results better than those of separate fluorescence and reflectance analyses on the germ side, as well as a clear improvement of the TPR. Aflatoxin concentration in corn was reduced from 2662.01 μg/kg to 64.04 μg/kg, 87.33 μg/kg and 7.59 μg/kg after removal of contaminated kernels using fluorescence, reflectance, and integrated analyses, respectively. These results suggest that the integration of fluorescence and reflectance HSI technique provides better classification of aflatoxin-contaminated kernels and leads to rapid and non-destructive scanning-based in-line sorting for the corn industry.

In a different approach, Wang et al. (2014) applied an SWIR HSI system in the 1000–2500 nm to assess its potential use for

Table 1. Hyperspectral imaging for detection of aflatoxin in food.

Aflatoxin Range (μg/kg)	Product	Mode	Spectral range (nm)	Optimal wavelengths (nm)	Data analysis	Accuracy	Reference
0–14000	Maize	Fluorescene	400–600	/	MLR	$R^2_P = 0.72$	Yao et al. (2010)
0–2000	Maize	Fluorescene	400–700	/	/	Correct classification rate was 94.4%	Yao et al. (2013a)
0–11000	Maize	Fluorescene	400–900	437 and 537	BE	Correct classification rate was 87% and 88% for 20 and 100 μg/kg threshold, respectively	Yao et al. (2013b)
0–8000	Maize	Reflectance	400–900	501 and 478	/	/	Hruska et al. (2013)
10–500	Maize	Reflectance	1000–2500	/	PCA FDA	Correct classification rate was > 88%	Wang et al. (2014)
0–3800	Maize	Reflectance	1000–2500	/	PCA	Correct classification rate was 92%	Wang et al. (2015)
0–1000	Maize	Reflectance	1100–1700	/	PLS	Correct classification rate was 97%	Kandpal et al. (2015)
0–2662	Maize	Fluorescene Reflectance	399–701 461–877	501 701	LS-SVM	Correct classification rate was 90.00%-95.33%	Zhu et al. (2016)

MLR = Multiple linear regression, PLS = Partial least sequares, BE = Binary encoding, PCA = Principal component analysis, R^2_P = Determination coefficient for prediction, FDA = Factorial discriminant analysis, LS-SVM = Least squares support vector machines.

detecting aflatoxin B_1 (AFB_1) artificially applied onto the surface of healthy maize kernels. A minimum classification accuracy of 88% was achieved for the validation set and verification set. This result indicates that SWIR HSI technology, accompanied by principal component analysis (PCA) and factorial discriminant analysis (FDA) method, could be used to detect AFB_1 applied directly on the maize surface at concentrations as low as 10 μg/kg. Similarly, Kandpal et al. (2015) used an SWIR HSI technique in the 1100–1700 nm range to detect AFB_1 artificially applied to the surface of healthy maize kernels. A partial least-squares discriminant analysis (PLS-DA) model was developed to categorize the control and infected kernels, and the highest overall classification accuracy yielded from the developed model was 96.9%. When the AFB_1 concentrations were increased, spectral deviation was observed between the control and contaminated samples. Additionally, the distribution of infected samples was observed by using a contamination map generated through the PLS-DA model. The results were interesting and meant that it is possible to discriminate between various concentrations of AFB_1 in asymptomatic kernels, even when the level was as low as 10 μg/kg. Further study on the feasibility of detecting the AFB_1 in maize kernels inoculated with *A. flavus* conidia in the field was evaluated using SWIR HSI technique in the 1000–2500 nm by Wang et al. (2015). They found that 1729 nm and 2344 nm can be used to characterize AFB_1 exclusively. In contrast to the verification accuracy of chemical analysis, that of SWIR HIS technique reached 100%, although it falsely identified the tip parts of healthy kernels to be contaminated with aflatoxin. Moreover, another 26 maize kernels were selected for the independent data set in order to verify the reproducibility of the method, and a high detection accuracy of 92.3% was achieved. Although the results suggest that SWIR HSI is a rapid, accurate, and non-destructive technique for the detection of aflatoxins in grains and is an alternative to the manual technique, further studies on the feasibility of this method are still necessary.

All the results suggested that hyperspectral imaging-based technique could be applied to classify aflatoxin contaminated food products and healthy kernels. However, most of these studies involved unrealistically high aflatoxin concentrations or artificially inoculated samples. The aflatoxin concentrations in these samples were much higher than the real concentrations in food products and the acceptable limits (2-20 μg/kg) in many countries. Therefore, the conclusions of these studies cannot be extrapolated to real situations. Further studies on the feasibility of hyperspectral imaging using the natural aflatoxin-contaminated food products should be carried out.

Applications of HSI for classification of aspergillus, penicillium and trichoderma contaminated food

Aspergillus and *Penicillium* are two common fungi that cause deterioration of food products such as oil and cereal grains. *Aspergillus* is a genus of ascomycetous fungi and consists of a few hundred mold species. *Aspergillus* species are common contaminants of oily and starchy foods (Singh, et al., 2011). Similar to *Aspergillus, Penicillium* is also a genus of ascomycetous fungi and frequently causes food spoilage during production (Zhang et al., 2014). Some species of this genus can produce toxic and carcinogenic secondary metabolites, such as patulin, citrinin, and penicilic acid, when they infect food and feed. It is necessary to detect such spoilage fungi by advanced techniques like HSI. Detailed information on use of HSI for the evaluation of *Aspergillus* and *Penicillium* contamination in food products is summarized and shown in Table 2. Yao et al. (2008) applied an HSI system with reflectance spectrum in the

Table 2. Hyperspectral imaging for detection of *Aspergillus, Penicillium* and *Trichoderma* in food.

Contaminant	Product	Mode	Spectral range	Optimal wavelengths	Data analysis	Accuracy	Reference
A. flavus *A. parasiticus* *Penicillium chrysogenum* *Fusarium verticillioides* *Trichoderma viride*	PDA media	Reflectance	400–1000 nm	743, 158 and 541 nm	ML	Correct classification rate was 97.7%	Yao et al. (2008)
Toxigenic *A. flavus* Atoxigenic *A. flavus*	PDA media	Reflectance	400–1000 nm		SVM	83% and 74% for toxigenic and atoxigenic *A. flavus* under halogen light 67% and 85% for toxigenic and atoxigenic *A. flavus* under UV lights	Jin et al. (2009)
A. flavus *A. parasiticus* *A. niger*	Maize	Reflectance	400–1000 nm	410, 470, 535 and 945 nm	PCA	/	Del Fiore et al. (2010)
A. glaucus *A. niger* *Penicillium* spp.	Wheat	Reflectance	700–1100 nm	/	LDA	Correct classification rate was 93.3-100%	Singh et al. (2012)
Aspergillus oryzae	Rice	Reflectance	400–1000 nm	/	PLS	$R^2_P = 0.97$ RMSEP = 0.39	Siripatrawan and Makino (2015)
Toxigenic *A. flavus* Atoxigenic *A. flavus*	Peanut	Fluorescene	400–900 nm	/	MAV	100% between inoculation group and control ~80% between two inoculation groups	Xing et al. (2017)
A. flavus	Date fruit	Reflectance	960–1700 nm	/	LDA QDA	Correct classification rate was > 90%	Teena et al. (2014)

LDA = Linear discriminant analysis, MAV = Multivariate analysis of variance, ML = Maximum Likelihood, PLS = Partial least sequares, PCA = Principal component analysis, QDA = Quadratic discriminant analysis, SVM = Support vector machine.

Table 3. Hyperspectral imaging for detection of *Fusarium* in food.

Contaminant	Product	Mode	Spectral range	Optimal wavelengths	Data analysis	Accuracy	Reference
Fusarium culmorum	Wheat	Reflectance	400–1000 nm	665-675 nm and 550–560 nm	PCA	Correct classification rate was 87%	Bauiegel et al. (2011)
Fusarium	Wheat	Reflectance	400–1000 nm	484, 567, 684, 817, 900 and 950 nm	PCA	Correct classification rate was over 92%	Shahin and Symons (2011)
Fusarium	Wheat	Reflectance	400–1000 nm	/	PLS-DA	Correct classification rate was 90%	Shahin and Symons (2012)
Fusarium	Wheat	Reflectance	400–1700 nm	1200 nm	LDA	Correct classification rate was 95%	Delwiche et al. (2011)
Fusarium	Wheat	Reflectance	400–790 nm	/	PLS-DA	Correct classification rate was 86%	Menesatti et al. (2013)
Fusarium	Wheat	Reflectance	1000–1700 nm	1209-1230 nm, 1489–1510 nm and 1601–1622 nm	PLS	RMSECV < 0.045	Serranti et al. (2013)
Fusarium verticillioides	Maize	Reflectance	400–1000 nm	410, 470, 535 and 945 nm	PCA	/	Del Fiore et al. (2010)
Fusarium graminearum *F. verticillioides*	Maize	Reflectance	1000–2498 nm	/	PLS	$R^2_P = 0.72$ RMSEP = 9.9	Williams et al. (2012)

PLS = Partial least sequares, PCA = Principal component analysis, LDA = Linear discriminant analysis, R^2_P = Determination coefficient for prediction, PLS-DA = Partial least squares discriminant analysis, RMSECV = Root mean square error of cross validation, RMSEP = Root mean square error of prediction.

400–1000 nm to differentiate fungal species (*Penicillium chrysogenum, F. verticillioides, A. parasiticus, Trichoderma viride*, and *A. flavus*) on potato dextrose agar (PDA) media. These species can be differenced under visible spectroscopy (VIS) by using only three narrow bands centered at 743 nm, 458 nm, and 541 nm. For all five fungi, high classification accuracy of 97.7% was obtained. Jin et al. (2009) applied the same system to classify the toxigenic and atoxigenic strains of *A. flavus*. By using a support vector machine (SVM) classifier, they correctly classified an average of 83% of the toxigenic-fungus pixels and 74% of the atoxigenic-fungal pixels under halogen light sources; while 67% of the toxigenic-fungus pixels and 85% of the atoxigenic-fungus pixels under UV lights. The pair-wise classification accuracies were 80%, 91% and 95% under halogen light sources between toxigenic AF13 and each atoxigenic strains (AF38, AF283 and AF2038), and 75%, 97% and 99% under UV lights, respectively. Del Fiore et al. (2010) applied the VNIR HSI system (400-1000 nm) to detect toxigenic fungi (*A. flavus, A. parasiticus, A. niger* and *Fusarium* spp) on maize kernels, and to discriminate healthy and diseased kernels. They found that HSI is able to rapidly discriminate commercial maize kernels infected with toxigenic fungi from uninfected controls when traditional methods are not yet effective: i.e. from 48 h after inoculation with *A. niger* or *A. flavus*. Singh et al. (2012) used a short-wave NIR HSI system in the 700–1100 nm wavelength range to detect fungus-damaged wheat kernels artificially infected with fungi (*Penicillium sp*p., *A. glaucus*, and *A. niger*). Linear discriminant analysis (LDA) showed an overall accuracy of 97.3%-100% in classifying the wheat kernels into healthy and fungi-infected categories. Siripatrawan and Makino (2015) used a visible HSI system in the 400–1000 nm wavelength range to detect *Aspergillus oryzae* artificially introduced to brown rice. By using PLS analysis, a very high correlation (R^2_P of 0.97 with RMSEV of 0.39 log CFU/g) between spectra and *A. oryzae* counts was obtained. This result indicates the advantage of HSI in the prediction of fungal infection in the early stage. Xing et al. (2017) applied a fluorescence VNIR HSI system in the 400–900 nm to classify peanut kernels inoculated with toxigenic and atoxigenic *A. flavus* and healthy kernels. MAV showed that the inoculated group can be separated from the controls with 100% accuracy. However, the two inoculation groups (AF3357 and AF36) can be separated with only ~80% accuracy. Date fruits (*Phoenix dactylifera*) inoculated with *A. flavus* were classified by HSI technique in the 960–1700 nm range (Teena et al., 2014). By LDA and quadratic discriminant analysis (QDA), mean classification accuracy was found to be over 91.0%–100%, with QDA being better than LDA in all classification models tested.

Applications of HSI for classification of fusarium-infected and healthy cereal

HSI is a promising technique that has been studied and introduced for the detection of *Fusarium* contamination of wheat and maize. An HSI system in four different wavelength ranges was used to differentiate artificially contaminated (head blight disease, artificially inoculated with *Fusarium culmorum*) and healthy ear tissues of wheat (Bauriegel et al., 2011). PCA indicated that the two ranges of 665–675 nm and 550–560 nm are suitable for head blight recognition, and mean hit rates were 67% during the whole study period. A VNIR HSI system in the 400–1000 nm was used to detect naturally *Fusarium*-damaged kernels of Canada Western Red Spring wheat (Shahin and Symons, 2011). LDA was applied for classification and the overall accuracy of ≥ 92% was achieved between healthy and infected wheat kernels. In another study, Shahin and Symons (2012) used the same VNIR HSI system to detect naturally *Fusarium*-infected kernels of seven Canadian wheat classes. PLS-DA was applied to *Fusarium* detection, and 90% overall accuracy and 9% false positives were obtained. Similarly, Delwiche, Kim and Dong (2011) used VNIR HSI in the 400–1700 nm range to detect naturally *Fusarium*-damaged kernels of American wheat. LDA was applied in the classification, and an overall accuracy of 95% was obtained. Furthermore, the spectral absorption near 1200 nm in the NIR region, which is attributed to ergosterol (a primary component of fungal cell membranes), was useful in the spectral recognition of *Fusarium* damage. In addition, the performance of VNIR imaging

spectroscopy (400–790 nm) for the early detection of *Fusarium* head blight infection of two cultivars of durum wheat (Creso and Simeto) was evaluated and compared in laboratory and in-field experiments (Menesatti et al., 2013). A classification accuracy of 86% was observed in the lab experiment, higher than that observed in the in-field experiment. This result implies that both approaches, especially the in-field experiment, still require further investigations in order to validate the techniques and to increase the robustness of the method. Serranti, Cesare and Bonifazi. (2013) used an NIR HSI system in the 1000–1700 nm range coupled with different chemometric techniques (PCA, PLS-DA and interval PLS-DA) to classify *Fusarium*-damaged, yellow berry and vitreous Italian durum wheat kernels. Good classification results were achieved with the entire investigated wavelength range and with only three narrow intervals of wavelengths (1209–1230 nm, 1489–1510 nm and 1601–1622 nm) out of 121. For maize, Del Fiore et al. (2010) used HSI techniques in the VNIR spectral range (400–1000 nm) to detect toxigenic fungi in artificially contaminated maize and to discriminate healthy and infected kernels. Their results show that HSI rapidly discriminates commercial maize kernels infected with toxigenic fungi from uninfected control, unlike traditional methods. Williams et al. (2012) used a near infrared HSI system (1000–2498 nm) to track changes in fungal contamination and fungal activity immediately under the surface of whole maize kernels infected with *F. verticillioides*. They found that this system can monitor changes in fungal infected maize kernels over time. PCA models showed three distinct clusters, making it possible to discriminate between the control and the samples 17 h and 20–90 h after inoculation. Variables identified as vital to this study are 1405 nm (associated with starch), 1660–1608 nm (associated with an aromatic structure), 1900 nm (associated with starch), and 2136 nm (associated with protein). All of the above studies suggest that HSI techniques could be utilized at the industrial level for safety control purposes or for the definition of innovative sorting logic for cereal kernels.

Conclusions

Because of their rapid response and ease of operation, spectral-based techniques have been studied in the past decade for the detection, classification and sorting of mycotoxins and toxigenic fungi in food products. Raman spectroscopy, FTIR spectroscopy and HSI have been considered as potential tools for rapid and non-destructive classification of various mycotoxins and toxigenic fungi in different food products. However, Raman spectroscopy and FTIR can presently analyze only a relatively small amount of ground food products. Therefore, the two methods are not "real" non-destructive detection tools. In contrast, HIS, which combines the major features and merits of spectroscopy and imaging technologies, provides both spectral and spatial information simultaneously. An HSI-based technique can analyze whole kernels, and can be a real non-destructive practical detection tool. Despite the many studies on mycotoxins and toxigenic fungi in food products, HSI still faces many challenges. The results of all the reported studies were obtained under static conditions. So, more researches are needed to improve the robustness of correlation models, to develop techniques that reduce hyperspectral image dimensionality, and to design cost-effective and efficient multivariate algorithms that enhance algorithm performance. In addition, works are also needed to standardize the procedure of HSI in terms of data mining, and multispectral imaging systems need to be developed for specific commodities to achieve real-time data acquisition and processing. Compact and portable instruments also need to be developed for daily applications, as well as instruments for online inspection. Lastly, other rapid and non-invasive detection techniques such as analysis using electronic nose and thermal imaging may be combined with spectral-based methods to improve their effectiveness in contamination detection. This combined sensor approach has the advantages of different types of instruments and compensates for the detection limits of each.

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