Spectroscopic Techniques for Nondestructive Detection of Fungi and Mycotoxins in Agricultural Materials: A Review

Hyunjung Min, Byoung-Kwan Cho*

Department of Biosystems Machinery Engineering, College of Agricultural and Life Science, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 305-764, South Korea

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Abstract

Purpose: Fungal secondary metabolite (mycotoxin) contamination in foods can pose a serious threat to humans and animals. Spectroscopic techniques have proven to be potential alternative tools for early detection of mycotoxins. Thus, the aim of this review is to provide an overview of the current developments in nondestructive food safety testing techniques, particularly regarding fungal contamination testing in grains, focusing on the application of spectroscopic techniques to this problem.

Methods: This review focuses on the use of spectroscopic techniques for the detection of fungi and mycotoxins in agricultural products as reported in the literature. It provides an overview of the characteristics of the main spectroscopic methods and reviews their applications in grain analysis.

Results: It was found that spectroscopy has advantages over conventional methods used for fungal contamination detection, particularly when combined with chemometrics. These advantages include the rapidness and nondestructive nature of this approach.

Conclusions: While spectroscopy offers many benefits for the detection of mycotoxins in agricultural products, a number of limitations exist, which must be overcome prior to widespread adoption of these techniques.

Keywords: Food safety, Fungi, Mycotoxins, Nondestructive measurement, Spectroscopy

Introduction

As the volume of agricultural commodity exports and imports continues to grow worldwide, food quality and safety are of great importance to the global economy and to human and animal health. In particular, mycotoxins, which are the product of fungal infection in agricultural products, have affected approximately 25% of the global food supply. Thus, interest in research towards the detection of contaminated food and feed grains has steadily increased. Many countries have imposed regulations on mycotoxin contamination in order to protect human health, as well as to prevent economic losses (Hussein and Jeffrey, 2001). The natural mycotoxins found in agricultural products are secondary metabolites produced by fungi. Once visible fungi are formed at any stage of the product processing (Jouany, 2007), the product can be easily colonized; this colony growth then influences nearby grains. Certain mycotoxins that are produced by fungi pose a threat to both humans and animals (Leslie et al., 2008). Further, mycotoxins are heat-resistant and their compounds are chemically stable; thus, it is difficult to eradicate them. They can easily enter the human food chain via meat from livestock that has consumed infected grains, which are impossible to detect through direct observation (Bryden, 2012). Therefore, methods for the rapid detection of mycotoxins in the early developmental stages are required. To date, research into qualitative and quantitative analysis methods for mycotoxin detection has been conducted extensively (Krska et al., 2008). However, these methods have certain limitations, and therefore, the development of effective detection methods remains a challenging task.

Since mycotoxins are diverse and can exist in small...
quantities, their accurate detection is essential. Over several decades, chromatographic methods have been the standard method for the detection of mycotoxins. There are a number of pre-treatment methods in chromatographic analysis, including extraction and clean-up purification. According to Turner et al. (2009), biological sample pre-treatment methods include liquid-liquid extraction (LLE), supercritical fluid extraction (SFE), and solid-phase extraction (SPE). For the analysis itself, liquid chromatography (LC) in combination with mass spectrometry (MS) is common. Other methods include thin layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), and capillary electrophoresis. Recently, the use of enzyme-linked immunosorbent assays (ELISA) and biosensors (Sapsford et al., 2006) has emerged in the chemical, pharmaceutical, and agricultural industries. However, the methods listed above have many disadvantages, as shown in Table 1 (Turner et al., 2009; Koppen et al., 2010).

Nondestructive measurement techniques have been developed to overcome the disadvantages of the conventional invasive methods listed above; the most widely used technique is spectroscopy (Herrero, 2008; Alishahi et al., 2010; Saeger, 2011). The principles of spectroscopic measurement are related to the molecular binding behavior in a specimen in response to electromagnetic radiation, and spectroscopic analysis has been used in many industries as an analytical tool to obtain both qualitative and quantitative information. Spectroscopic techniques are based on the absorption, emission, and scattering of light interacting with a specimen over a broad wavelength range. Christopher (1997) has listed various spectroscopic techniques that have been used for food analysis: nuclear magnetic resonance (NMR) spectroscopy, electron paramagnetic resonance (EPR) spectroscopy, electron spin resonance (ESR) spectroscopy, dielectric spectroscopy (DS), mid-infrared (MIR) and near-infrared (NIR) spectroscopy, photoacoustic spectroscopy (PAS), Raman spectroscopy, optothermal NIR spectroscopy, ultraviolet (UV)/visible spectroscopy, circular dichroism (CD) spectroscopy, and X-ray spectrometry. Of these spectroscopic techniques, visible-NIR (VIS/NIR), short-wave infrared (SWIR), MIR, far infrared, UV, Raman, and fluorescence spectroscopic techniques have been used for mycotoxin detection.

At present, the hyperspectral imaging (HSI) technique is used as an alternative spectroscopic point measurement technique for the rapid measurement of large samples of materials. HSI obtains images over a broad wavelength range with a narrow spectral interval, while multispectral imaging provides spectral images with much wider spectral intervals. Thus, the HSI system is an advanced inspection tool for the detection of defects in food. Through the line-scanning method, it is possible to use consecutive wavelengths to create a 3D hyper-cube, which consists of 2D images combined with 1D spectral information for each pixel in the images. Thus, as it is composed of hundreds of continuous wavelengths, HSI offers advantages; it can simultaneously present spectral and spatial information of an object (Lorente et al., 2012). The HSI technique has been applied to the detection of defects and contaminants in a variety of agricultural materials, such as wheat (Singh et al., 2009a; Shahin and Symons, 2011; Singh et al., 2012b), corn (Cogdill et al., 2004; Williams et al., 2009; Williams et al., 2012b), fruits (ElMasry et al., 2007), vegetables (Ariana et al., 2006; Cho et al., 2013), and meat (Qiao et al., 2007; Kamruzzaman et al., 2012). Multispectral imaging techniques have also been used for the detection of mycotoxins in grain (Firrao et al., 2010; Kalkan et al., 2011; Montalban et al., 2011).

The objective of this review was to investigate the current progress of the development of nondestructive measurement techniques for food safety testing, especially in regards to the detection of fungal contaminants in grains, focusing on spectroscopic techniques and chemometrics.

**Fungal contaminants (mycotoxins)**

Mycotoxins, which are secondary metabolites that are produced naturally by fungi, are harmful to humans and animals. Mycotoxins develop in grain in response to environmental factors: for example, specific moisture, pH, and temperature conditions. Mycotoxins absorbed by humans and animals can cause health disorders such as liver and kidney damage. Hence, the sale of grain containing mycotoxins is strictly prohibited by law (Egmond et al., 2007).

Three types of mycotoxins originate from the toxigenic fungi: *Aspergillus, Penicillium,* and *Fusarium* (Sweeney et

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Table 1. Limitations of conventional methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>Laborious</td>
</tr>
<tr>
<td>TLC</td>
<td>Large quantities of solvent</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Expensive equipment</td>
</tr>
<tr>
<td>GC</td>
<td>Complex cleaning of samples</td>
</tr>
<tr>
<td>ELISA</td>
<td>Time-consuming</td>
</tr>
<tr>
<td>Biosensor</td>
<td>Insensitive</td>
</tr>
</tbody>
</table>
The aflatoxin and ochratoxin mycotoxins are produced by *Aspergillus*, while patulin and citrinin are produced by *Penicillium*. The fumonisin, T-2, deoxynivalenol (DON), and zearalenone mycotoxins are produced by *Fusarium*.

Aflatoxins are now known to be produced by two closely related species of mold, *Aspergillus flavus* and *A. parasiticus*, and aflatoxins in peanuts caused the deaths of 100,000 turkeys in the United Kingdom in 1960 (Newberne and Butler, 1969). Of the B1, B2, G1, and G2 aflatoxin varieties, aflatoxin B1 has the highest toxicity risk; thus, it is considered a Group 1 carcinogen. It can also cause acute hepatitis in humans, according to the International Agency for Research on Cancer (IARC, 1993a). Aflatoxins can also cause teratogenesis and act as nephrotoxins. They can be found in common food commodities such as maize, wheat, and rice (Natalia et al., 2014).

Ochratoxins (A, B, and C) are metabolites produced by *Penicillium verrucosum* and *A. ochraceus* (Creppy, 2002). Ochratoxin A is a potent nephrotoxin and has been found in coffee, nuts, corn, rice, and wheat (Höhler, 1998; Petzinger and Weidenbach, 2002). It is also a Group 2B carcinogen, as determined by the International Agency for Research on Cancer (IARC, 1993b). Fumonisin (B1, B2, B3, B4, A1, and A2) has a global distribution and contaminates corn, rice, and wheat (Munkvold and Desjardins, 1997). Fumonisin B1 is most frequently found in agricultural commodities, and the European Commission has set the standard for the acceptable level of fumonisin at 100-400 mg/kg in corn. Fumonisin B1 was classified as a Group 2B carcinogen by the International Agency for Research on Cancer (IARC, 2002), and can be both hepatotoxic and carcinogenic to humans (Placinta et al., 1999).

The T-2 toxin is produced by *Fusarium* species. T-2 toxins can induce acute and chronic toxicity leading to mutagenesis and neurotoxicity, and are found in wheat, barley, and rice, among others (D’Mello and Macdonald, 1997). Deoxynivalenol (DON) is a secondary metabolite produced by *Fusarium*, and is predominantly found in barley, maize, oats, and wheat. DON contamination results in acute illness involving nausea and vomiting (Pestka, 2007). Zearalenone has been found in most cereals, but has relatively low toxicity (Zinedine et al., 2007). However, this toxin induces abnormal estrogenic activity. In short, it is apparent that the majority of mycotoxins pose serious threats to health owing to their carcinogenic properties. They can cause chronic, acute, and potentially fatal illness in humans and animals.

Mycotoxins are not only dangerous, but also invisible to the naked eye; thus, mycotoxin detection is both challenging and important. In general, chromatographic methods are used to detect grain contamination by mycotoxins. However, because of the many disadvantages of chromatographic methods as outlined above, nondestructive measurement techniques utilizing spectroscopy have recently been developed for the rapid monitoring and detection of mycotoxin contamination in grain (Williams et al., 2012a).

**Principles and application of spectroscopy**

Several spectroscopic methods, such as NIR, Raman, fluorescence, and HSI techniques, have been used for the nondestructive measurement of food and agricultural materials. In this section, the principles of several spectroscopic techniques will be briefly introduced.

Firstly, IR radiation can be divided into the near-, mid-, and far-infrared regions, which are located in the 700~2,500, 3,000~5,000, and 8,000~12,000 nm wavelength ranges, respectively. The characteristics of the IR spectrum are used in a number of different measurement methods including transmission spectroscopy, microspectroscopy, specular reflectance, photoacoustic spectroscopy (PAS), diffuse reflectance (DR), and attenuated total reflectance (ATR) (Mariey et al., 2001).

The most widely used spectroscopic technique for the analysis of agricultural materials is NIR spectroscopy (NIRS), and its modes are transmittance, interactance, transfectance, diffuse transmittance, and DR (Huang et al., 2008). NIR wavelengths have higher light transmittance, so they provide higher-resolution information than does MIR light. Many studies have been conducted to apply NIRS to quality and safety measurements for food and agricultural materials, including fruits and vegetables (Nicolai et al., 2007), and food and beverages (Huang et al., 2008). Magwaza et al. (2012) have reviewed applications of NIRS to the internal and external analysis of citrus-containing materials. Roggo et al. (2007) have also reviewed NIRS applications in the pharmaceutical industry.

Raman spectroscopy utilizes scattered light of different wavelengths following the irradiation of the incident light on a sample. When the light irradiates a specimen, the energy is absorbed, reflected, and partially scattered by the sample. Various types of scattering appear, including Raman and Rayleigh scattering, depending on whether the wavelength of emission of radiation is shorter or
longer than that of the incident light (Chan, 1996). Rayleigh scattering involves elastic scattering, while Raman scattering is inelastic. Surface-enhanced Raman spectroscopy (SERS), through which the sensitivity of the Raman signal is enhanced, was discovered in 1974. SERS utilizes colloid-based and solid surface-based substrates (Zheng and He, 2014), causing an increase in the Raman intensity and enabling the measurement to be conducted in a short time (Kneipp et al, 1997).

Fluorescence spectroscopy uses the emission spectrum of a material when it is exposed to shorter wavelength irradiation. Long-wave ultraviolet light (UV-A), laser sources, and light-emitting diodes (LEDs) are used as excitation sources for fluorescence measurement (Zhang et al, 2012). Fluorescence spectroscopy is applied to quality and safety measurements for a variety of food and agricultural materials, such as dairy products, meat, fish, eggs, edible oil, cereals, sugar, fruits, and vegetables (Vargas et al, 2005; Karoui and Blecker, 2011). However, this technique has several limitations, such as strong dependence on auto-fluorescence of samples and on the intensity of incident light (Zhang et al, 2012).

The HSI system has been more widely used for food quality and safety. It is based on a combination of imaging and spectroscopic measurements that can be feasibly applied to the rapid measurement of a large amount of samples. Reflectance, transmission, and emission measurement modes can be used in different applications (Gowen et al, 2007). Mehl et al. (2004) have produced images of contaminated and defective apples using HSI techniques, while Wang et al (2012) have investigated the feasibility of SWIR HSI for the detection of contaminants in onions using a support vector machine (SVM) classifier with 87.1% classification accuracy. Bauriegel et al. (2011) have focused on the detection of Fusarium-infected wheat and used spectral angle mapping (SAM) as their analysis method.

**Chemometrics**

Chemometric analysis is a multivariate statistical technique used for the interpretation of spectral data (Geladi, 2003; Adams, 2004; Varmuza and Filzmoser, 2009). It is difficult to analyze spectral data from complex functional groups of agricultural materials directly because they contain many superposed overtones and combination bands. However, through the extraction of appropriate information from data sets, it is possible to interpret spectroscopic data (Rossel, 2008). This enables researchers to identify chemical structures and determine the chemical compound concentrations in agricultural materials. When measuring samples, external environmental factors such as illumination and temperature must be considered. To eliminate undesirable effects from the external environment, preprocessing must be conducted prior to chemometric analysis. Several preprocessing methods, such as averaging, centering, smoothing, standardization, normalization, transformation, multiplicative scatter correction (MSC), and standard vector normalization (SNV) have been used (Nicolai et al, 2007; Cen and He, 2007). The most frequently used multivariate statistical techniques are principal component analysis (PCA), partial least-squares (PLS), linear discriminant analysis (LDA), and artificial neural networks (ANN) (Lorente et al, 2012). These classification techniques can be divided into two categories: unsupervised and supervised analyses. PCA is an unsupervised method, while PLS, LDA, and ANN are supervised methods (Roggo et al, 2007).

**Application in grains**

Of the spectroscopy techniques, IR, Raman, NIR, NMR, and UV spectroscopy have been used for mycotoxin detection (Saeger, 2011). In particular, NIRS has been widely used in combination with multivariate statistical methods. Berardo et al. (2005) have measured fungal infections, focusing on the development of fumonisin B1 and ergosterol using NIRS. Sirisomboon et al. (2013) have applied NIRS (950~1,650 nm) to the detection of aflatoxigenic fungal infections in rice. The observed samples included both naturally and artificially infected rice with different levels of fungal infection. Ibanez et al. (2009) used both an NIR system with a 400~2,500-nm range and a Fourier-transform (FT)-NIR system with a range of 1,112~2,500 nm for aflatoxin B1 detection; FT-NIR extracted more detailed spectral information than did dispersive NIR spectroscopy. NIR transmittance has also been used for DON detection in cereals (Pettersson and Aberg, 2003). For example, Peiris et al. (2009) estimated DON levels in Fusarium-damaged kernels using NIR along with a VIS/NIR spectrometer, while Girolamo et al. (2014) reported the application of FT-NIR spectroscopy to DON detection in durum wheat. PCA, three LDA models, and PLS models were used for analysis (Girolamo et al, 2014). The ability of PLS classification models was lower than the ability of LDA classification models. Gaspardo et al. (2012) and Giacomo...
and Stefania (2013) used FT-NIR spectroscopy for the detection of fumonisin in corn meal and maize, respectively. Giacomo and Stefania (2013) showed that their obtained calibration and prediction plot emphasized the 4 mg kg\(^{-1}\) threshold that is stipulated by EU regulation.

IR spectroscopy has proven to be a suitable approach to safety measurement in grains. Bozza et al. (2013) employed FT-IR to detect ochratoxin A and compared the results obtained by reflectance and transmittance measurements. Different types of infrared spectroscopy, such as specular reflectance, PAS, DR, and ATR have also been used (Stuart, 2004). Kos et al. (2003) used MIR ATR spectroscopy to detect ergosterol and DON, while another study used MIR with ATR and DRS for the detection of DON and ergosterol (Kos et al., 2004). This study demonstrated that ATR-based classification is more effective than DRS-based classification. Abramović et al. (2007) also compared the DR and ATR techniques in the detection of DON in wheat, and showed that ATR has several advantages in classification and quantification. In another approach, Gordon et al. (1999) used Fourier-transform infrared photoacoustic spectroscopy (FTIR-PAS) and transient infrared spectroscopy (TIRS) for the detection of *A. flavus* infection in corn. TIRS uses thermal energy to heat or cool sample surfaces and has many advantages; however, sample size is limited in this technique.

The HSI Vis/NIR technique has been used for the detection of defective and contaminated products. For example, Jin et al. (2009) have demonstrated the classification of toxigenic and atoxigenic strains using a Vis/NIR HSI system. In that study, halogen and UV light sources were used, and it was found that the classification obtained under UV light was more accurate than under halogen light. Yao et al. (2008) prepared toxigenic fungi for mold separation and revealed that two types of fungi could be classified using HSI. Fiore et al. (2010) detected fungi-inoculated maize kernels using Vis/NIR HSI with a spectral range of 400–1,000 nm in the reflectance mode, while Shahin and Symons (2011) used HSI for the detection of *Fusarium* in damaged maize kernels. Wang et al. (2014) used SWIR HSI in combination with the PCA-Factorial discriminant analysis (FDA) method to distinguish between different levels of aflatoxin B\(_1\), while Wang et al. (2015) demonstrate that the 1,729 nm and 2,344 nm peaks were due to the presence of aflatoxin B\(_1\). Further, the results indicated the possibility of differentiating between varying levels of aflatoxin B\(_1\) ranging from pure to approximately 3,800 ppb on the maize surface. Williams et al. (2012c) demonstrated the possibility of identifying *Fusarium* spp. using NIR HSI combined with the PCA and PLS-DA methods.

In regards to Raman spectroscopy, Lee et al. (2014a) revealed that maize samples contaminated by aflatoxin could be distinguished from uncontaminated samples. A Raman system equipped with a 350-mW 785-nm NIR laser was used in that study. Several preprocessing methods, including normalization, first derivative, second derivative, and deconvolution techniques, were applied to optimize the classification, and the LDA method turned out to be the most successful discriminant model. In addition, Lee et al. (2014b) studied the feasibility of SERS with Ag nanospheres (NS) for the detection of aflatoxin contamination in maize, as SERS had significantly higher sensitivity than did Raman spectroscopy. Multi-linear regression (MLR), principal component regression (PCR), and partial least-squares regression (PLSR) were used for the development of aflatoxin quantification models with SERS. MLR analysis was found to provide a highly satisfactory outcome. However, SERS has disadvantages associated with nanoparticle instability.

Yao et al. (2010) used a hyperspectral fluorescence imaging (HSFI) system to detect aflatoxin-contaminated corn; a UV-A lamp was used as a fluorescence excitation light source. The obtained results were analyzed using SAM as a supervised classification. It was found that the classification accuracy was 86.1% for 20 ppb of aflatoxin and 88.1% for 100 ppb. Zhang et al. (2012) observed that HSFI requires considerable time to obtain crucial features. In addition, Yao et al. (2013) directly inoculated maize with toxigenic and atoxigenic fungal strains. After imaging the maize kernels contaminated with the fungal strains, it was found that the germ component of the maize kernels yielded superior imaging results compared to the endosperm. The measurement was performed using an HSFI system containing UV-A lamps with central wavelengths of 365 nm. The imaging data were analyzed using discriminant analysis.

Tables 2 and 3 summarize the applications of spectroscopy techniques to the detection of fungi and mycotoxins in grains.

**Limitations**

Consumer concerns regarding food safety are growing because of the increasing appearance of hazardous materials in the production and distribution environments of agri-
cultural materials. Many studies are investigating rapid and precise measurement techniques for the detection of contaminated food materials. However, current nondestructive techniques for mycotoxin detection have several shortcomings. The detection limits of the nondestructive measurement of the various mycotoxins have not been identified. In addition, the instrument is expensive and its setup is complex. Moreover, nondestructive techniques such as spectroscopy are less accurate because they are influenced by external environmental factors such as temperature and light. As a result, the treatment of large data sets using these methods is time-consuming.

### Table 2. Application of spectroscopy to fungi and mycotoxin measurements

<table>
<thead>
<tr>
<th>Technique</th>
<th>Sample</th>
<th>Detection</th>
<th>Wavelength/wavenumber range</th>
<th>Chemometrics</th>
<th>Year</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTIR</td>
<td>Ochratoxin A</td>
<td>7,500-400 cm⁻¹</td>
<td>PLS</td>
<td>2013</td>
<td>[7]</td>
<td></td>
</tr>
<tr>
<td>Mid infrared</td>
<td>Corn</td>
<td>Fusarium graminearum</td>
<td>650-4,500 cm⁻¹</td>
<td>PCA, PLS, KNN</td>
<td>2003</td>
<td>[41]</td>
</tr>
<tr>
<td>FTIR-ATR, DR</td>
<td>Corn</td>
<td>F. graminearum</td>
<td></td>
<td>PCA, PLS, KNN</td>
<td>2004</td>
<td>[44]</td>
</tr>
<tr>
<td>FTIR-DRS, ATRS</td>
<td>Wheat</td>
<td>F. graminearum</td>
<td>650-4,000 cm⁻¹</td>
<td>MLR, PLS</td>
<td>2007</td>
<td>[1]</td>
</tr>
<tr>
<td>TIR, FTIR- PAS</td>
<td>Corn</td>
<td>A. flavus</td>
<td></td>
<td>PCA, PLS</td>
<td>1999</td>
<td>[24]</td>
</tr>
<tr>
<td>NIR, FTNIR</td>
<td>Maize, barley</td>
<td>Aflatoxin B1</td>
<td>400-2,500 nm, 1,112-2,500 nm</td>
<td>PLS</td>
<td>2009</td>
<td>[32]</td>
</tr>
<tr>
<td>NIR</td>
<td>Rice</td>
<td>A. flavus M3T8R4G3 aflatoxigenic strain</td>
<td>950-1650 nm</td>
<td>PLSR</td>
<td>2013</td>
<td>[69]</td>
</tr>
<tr>
<td>NIR</td>
<td>Maize</td>
<td>F. verticillioides</td>
<td>400-1100 nm</td>
<td></td>
<td>2005</td>
<td>[6]</td>
</tr>
<tr>
<td>NIR</td>
<td>Corn</td>
<td>fungal damage</td>
<td>900-1700 nm</td>
<td>LDA, MLP</td>
<td>2011</td>
<td>[72]</td>
</tr>
<tr>
<td>FTNIR</td>
<td>Maize</td>
<td>FB1 + FB2</td>
<td>650-2500 nm</td>
<td>PLS</td>
<td>2013</td>
<td>[22]</td>
</tr>
<tr>
<td>FTNIR</td>
<td>Wheat</td>
<td>DON</td>
<td>10000-4000 cm⁻¹</td>
<td>PLS, LDA</td>
<td>2014</td>
<td>[23]</td>
</tr>
<tr>
<td>FTNIR</td>
<td>Corn meal</td>
<td>FB1 + FB2</td>
<td>650-2500 nm</td>
<td>PLS</td>
<td>2012</td>
<td>[20]</td>
</tr>
<tr>
<td>NIRT</td>
<td>Wheat</td>
<td>DON</td>
<td>570-1100 nm</td>
<td>PLS, PCA</td>
<td>2003</td>
<td>[56]</td>
</tr>
<tr>
<td>Raman</td>
<td>Maize</td>
<td>Aflatoxins</td>
<td>785 nm</td>
<td>MLR, PCR, PLSR</td>
<td>2014</td>
<td>[44]</td>
</tr>
<tr>
<td>SERS</td>
<td>Maize</td>
<td>Aflatoxins</td>
<td>785 nm</td>
<td>MLR, PCR, PLSR</td>
<td>2014</td>
<td>[45]</td>
</tr>
</tbody>
</table>

### Table 3. Application of hyperspectral imaging systems to fungi and mycotoxin measurement

<table>
<thead>
<tr>
<th>Technique</th>
<th>Sample</th>
<th>Detection</th>
<th>Wavelength/wavenumber range</th>
<th>Chemometrics</th>
<th>Year</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWIR hyperspectral</td>
<td>Wheat</td>
<td>Penicillium spp., A. glaucus, A. niger</td>
<td>700-1100 nm</td>
<td>LDA, QDA, Mahalanobis</td>
<td>2012</td>
<td>[68]</td>
</tr>
<tr>
<td>SWIR hyperspectral</td>
<td>Maize kernel</td>
<td>Fusarium spp.</td>
<td>1000-2500 nm</td>
<td>PCA, PLS</td>
<td>2012</td>
<td>[80]</td>
</tr>
<tr>
<td>SWIR hyperspectral</td>
<td>Fusarium spp.</td>
<td></td>
<td>996-2502 nm</td>
<td>PCA</td>
<td>2012</td>
<td>[79]</td>
</tr>
<tr>
<td>SWIR hyperspectral</td>
<td>Fusarium spp.</td>
<td></td>
<td>1000-2498 nm</td>
<td>PCA, PLS-DA</td>
<td>2012</td>
<td>[81]</td>
</tr>
<tr>
<td>SWIR hyperspectral</td>
<td>Maize</td>
<td>Aflatoxin B1</td>
<td></td>
<td>PCA, SAM</td>
<td>2015</td>
<td>[77]</td>
</tr>
<tr>
<td>Visible NIR hyperspectral</td>
<td>Maize</td>
<td>Aspergillus strains, Fusarium spp.</td>
<td>400-1000 nm</td>
<td>PCA</td>
<td>2010</td>
<td>[18]</td>
</tr>
<tr>
<td>Visible NIR hyperspectral</td>
<td>Maize</td>
<td>Aspergillus strains</td>
<td>400-1000 nm</td>
<td>PCA</td>
<td>2009</td>
<td>[34]</td>
</tr>
<tr>
<td>Visible NIR hyperspectral</td>
<td>Wheat</td>
<td>Fusarium</td>
<td>400-1000 nm</td>
<td>PCA, LDA</td>
<td>2011</td>
<td>[66]</td>
</tr>
<tr>
<td>Visible NIR hyperspectral</td>
<td>Penicillium, Fusarium, Aspergillus, Trichoderma</td>
<td></td>
<td>400-1000 nm</td>
<td></td>
<td>2008</td>
<td>[84]</td>
</tr>
<tr>
<td>Visible NIR hyperspectral</td>
<td>Maize</td>
<td>Aflatoxin B1</td>
<td>1000-2500 nm</td>
<td>PCA-FDA</td>
<td>2014</td>
<td>[76]</td>
</tr>
<tr>
<td>Fluorescence hyperspectral</td>
<td>Maize</td>
<td>A. flavus</td>
<td>UV lamp: 365 nm</td>
<td>DA</td>
<td>2013</td>
<td>[68]</td>
</tr>
<tr>
<td>Fluorescence hyperspectral</td>
<td>Maize</td>
<td>A. flavus</td>
<td>UV lamp: 365 nm</td>
<td>SAM</td>
<td>2010</td>
<td>[85]</td>
</tr>
</tbody>
</table>
Conclusions

This paper discusses the feasibility of various spectroscopic methods for the detection of mycotoxins in grains. Mycotoxins are usually dangerous carcinogens that threaten human and animal life. Conventional methods are time-consuming and demanding; thus, spectroscopy could be a possible alternative means of detecting mycotoxins. There are a variety of spectroscopic techniques and each measurement method has respective advantages for the detection of mycotoxins in grains. A hyperspectral imaging system is also utilized to visualize the spectral characteristics of many samples simultaneously. It can be used for rapid measurement of massive amount of contaminated grains. Chemometric techniques combined with spectral data provide quantitative and qualitative analyses of samples. However, novel multivariate analysis methods would be necessary to increase the detection accuracy for low concentrations of mycotoxins in grains. Spectroscopic measurements for the detection of mycotoxins in grains have a number of advantages, but limitations exist as well. Further studies will be necessary to provide real-time monitoring and accurate mycotoxin concentration detection.

Conflict of Interest

The authors have no conflicting financial or other interests.

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