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The potential and applicability of infrared spectroscopic methods for the rapid screening and routine analysis of mycotoxins in food crops

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Abstract

Infrared (IR) spectroscopy is increasingly being used to analyze food crops for quality and safety purposes in a rapid, nondestructive, and eco-friendly manner. The lack of sensitivity and the overlapping absorption characteristics of major sample matrix components, however, often prevent the direct determination of food contaminants at trace levels. By measuring fungal-induced matrix changes with near IR and mid IR spectroscopy as well as hyperspectral imaging, the indirect determination of mycotoxins in food crops has been realized. Recent studies underline that such IR spectroscopic platforms have great potential for the rapid analysis of mycotoxins along the food and feed supply chain. However, there are no published reports on the validation of IR methods according to official regulations, and those publications that demonstrate their applicability in a routine analytical set-up are scarce. Therefore, the purpose of this review is to discuss the current state-of-the-art and the potential of IR spectroscopic methods for the rapid determination of mycotoxins in food crops. The study critically reflects on the applicability and limitations of IR spectroscopy in routine analysis and provides guidance to non-spectroscopists from the food and feed sector considering implementation of IR spectroscopy for rapid mycotoxin screening. Finally, an outlook on trends, possible fields of applications, and different ways of implementation in the food and feed safety area are discussed.

KEYWORDS
food safety, infrared spectroscopy, mycotoxin

1 | INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by fungi associated with agricultural commodities threatening the well-being of animals and humans (da Rocha et al., 2014). Besides their hazardous character, mycotoxins cause enormous economic losses worldwide. For wheat alone, €1.2–2.4 billion are lost per year in Europe (Krska et al., 2016). Recent research showcased that due to the omnipresence of these natural toxins, they can be found in up to 80% of analyzed food crops (Eskola et al., 2020). In fact, spoilage with mycotoxins renders 5–10 % of the global production of grains unusable as estimated by the European Union (Commission, 2015). Moreover, climate change will promote and change the occurrence of mycotoxigenic fungi. Contamination with aflatoxins (AFLAs) has already been reported as a future problem in southern Europe, due to global warming and extreme weather conditions. *Fusarium* species producing deoxynivalenol (DON) are advancing further into the northern hemisphere (Moretti et al., 2019). Consequently, the clearance of agricultural products intended for human and animal consumption remains a constant challenge. From the year 2010–2015, in Germany alone regarding mycotoxin contamination, 74,696 analyses of grain samples have been performed, according to the European Food Safety Authority (Eskola et al., 2020). To comply with mycotoxin regulations, the number of analyses is likely to further increase. It is clear, that cost-effective, rapid, simple but reliable methods are needed to accurately analyze this vast number of agricultural samples to ensure food and feed safety.

The discovery of AFLAs in the early 1960s launched the area of modern mycotoxicology (Pitt & Miller, 2017). Since then a wi collection of analytical methods have been developed for the determination of mycotoxins in food and feed (Bueno et al., 2015). These methods can be divided into sophisticated routine analytical techniques and less complex rapid screening approaches. Early analytical procedures were based on chromatographic separation techniques like high-performance liquid chromatography and gas chromatography coupled to fitting detector systems. These early routine analytical methods focused on the quantification of groups of relatively similar analytes, such as trichothecene mycotoxins. This usually involved tedious method development including sophisticated sample preparation to eliminate interfering matrix compounds, such as protein, fats, and carbohydrates (Krska et al., 2008). In the last two decades, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) evolved into the gold standard methodology for mycotoxin analysis (Malachová et al., 2018). LC-MS/MS enables fast extraction, minimal clean-up, multi analyte detection besides providing excellent sensitivity and selectivity. However, those LC-MS/MS–based methods require highly trained personnel and are expensive. A minimum sample handling time of at least 24 h, from sample arrival to the result, is required due to the labor intense and time-consuming character of these analytical techniques. This general drawback of chromatography-based analytical methods fueled the development of faster, mostly immunoanalytical methods for the rapid screening of mycotoxins. Enzyme linked immunosorbent assays (ELISA) are commercially available and routinely used for this purpose. Assessment of mycotoxin contamination can be performed with ELISAs in 0.5–2 h. Easy-to-use lateral flow assays (LFAs) allow for rapid semiquantitative screening with measurement times of 5–10 min excluding sample preparation time. Both approaches require only minimal sample preparation including homogenization and extraction due to the selectivity of the employed antibodies. However, both assay types are still relatively labor and consumable intense, suffer from cross-reactivity issues and matrix interferences, and require basic laboratory skills and equipment. (Bueno et al., 2015).

A wide variety of spectroscopic methods has been used for mycotoxin analysis including, infrared (IR), Raman, fluorescence, surface plasmon resonance and X-Ray spectroscopy, as well as imaging techniques (Bueno et al., 2015; Mishra et al., 2021). Among those, IR spectroscopy has a long history in the analysis of agricultural crops (Williams et al., 2019). IR spectrometers are relatively cheap compared to hyphenated techniques and only limited sample handling, few consumables, and often no solvents are required, rendering IR spectroscopy as a green analytical technique (Gałuszka et al., 2013). Operation of a routine IR spectrometer is straightforward allowing sample throughput times of 2 min, thus, reducing the cost per analysis down to a few dollars, once a proper calibration is established (Williams et al., 2019). In recent years, the potential and promising character of mycotoxin determination in agricultural products has been outlined by several authors (Jia et al., 2020; Levasseur-Garcia, 2018; McMullin et al., 2015; Orina et al., 2017).

In contrast to previous reviews, this manuscript provides a better fundamental understanding of common IR spectroscopic techniques with regards to mycotoxin determination in food crops. A comprehensive overview of the existing literature is provided, and advantages and limitations of current IR-methods are discussed with an emphasis on those papers that showcase the readiness of IR spectroscopy for mycotoxin routine analysis. The differences between mid IR (MIR) and near IR (NIR) spectroscopy, as well as hyperspectral imaging (HSI) and the consequences for implementation of these techniques for

mycotoxin screening will be discussed. A special focus on the applicability of the reported IR spectroscopic methods for mycotoxin analysis meeting regulatory requirements is presented. A workflow for implementing IR spectroscopy (i.e., model development) is proposed including, considerations on the selection of the most suitable approaches to be used for the IR spectroscopic set-up, reference analysis, sample collection, and data analysis. This review provides guidance for analytical scientists and food analysts aiming for the development and employment of IR spectroscopic methods for the screening of mycotoxins contamination in agricultural samples.

2 | INFRARED SPECTROSCOPY FOR MYCOTOXIN SCREENING

IR spectroscopy is based on the absorption of electromagnetic radiation having the same frequency as molecular vibrations (Colthup, 2012). The frequency of molecular vibrations is linked to the mass of the involved atoms and the bond strength between them, resulting in vibrational frequencies specific for functional groups. Consequently, IR spectroscopy can be used to obtain specific molecular information in a rapid and nondestructive manner (Colthup, 2012; Griffiths & De Haseth, 2007). Thus, IR spectroscopy is highly interesting for analyzing crops as the sample is not consumed by this technique; ideally, it can still be used as food or feed or for subsequent investigations. The IR region in the electromagnetic spectrum is located between the visible (Vis) and the terahertz region (Colthup, 2012). For screening of mycotoxins, the near-infrared (NIR) and the mid-infrared (MIR) of the overall IR region is used (Levasseur-Garcia, 2012). The NIR region ranges from 800 nm to 2.5 μm, followed by the MIR region, from 2.5 to 25 μm. In MIR spectroscopy (MIRS) spectra are plotted as a function of wavenumbers and therefore the MIR region is also defined to range from 4000 cm^{-1} to 400 cm^{-1} (Griffiths & De Haseth, 2007). Fundamental vibrations are probed in the MIR region; several harmonics or overtones as well as combination vibrations of fundamental vibrations are probed in the NIR region. Combinational bands are found over the whole NIR region, first overtones between 2000 to 1500 nm, second overtones between 2000 and 1000 nm, and the third overtones from 1500 to 800 nm (Williams et al., 2019; Workmann, 2012). This repetitive information allows for parts of the NIR region to be exploited, which has been showcased for the determination of mycotoxins or fungal infestation (Börjesson et al., 2007; Liang et al., 2020; Ruan et al., 2002; Williams et al., 2010). Compared to the MIR region, the information found in the NIR region is not only repetitive but also blurred, as NIR bands are broad and overlapping. This makes NIR spectra more difficult to interpret and the information linked to mycotoxin contamination hard to extract. The intensity of infrared absorption depends on the dipole moment change during molecular vibration. The dipole moment change of overtone vibrations is linked to the anharmonicity of molecular vibrations, resulting in small absorbance coefficients of overtone bands compared to their fundamental vibrations. Functional groups featuring a hydrogen atom have relatively large anharmonic constants, therefore absorption bands linked to CH, NH, and OH dominate NIR spectra (Colthup, 2012). Consequently, an NIR spectrum does not fully replicate the information found in the corresponding MIR spectrum. The intensity of overtone bands decreases by at least an order of magnitude with the order of the overtone. Due to the weak and broad bands within the NIR region, it has been neglected for a long time. However, the low absorbance coefficients of overtones are a critical advantage in real-life applications as more sample can be analyzed at once. This led to the widespread use of NIR spectroscopy (NIRS) for analyzing crops and other complex samples (Ciurczak et al., 2021).

The fundamental differences between NIRS and MIRS are reflected by the spectrometers used in the respective spectral region. In general, a spectrometer consists of four main parts: the radiation source, wavelength discrimination elements, a sampling interface, and a detector. Different radiation sources and detectors are used for NIRS and MIRS, respectively, as well as different sampling interfaces (Ciurczak et al., 2021; Griffiths & De Haseth, 2007). A wide variety of benchtop and portable NIR spectrometers exist, using optical filters or gratings to tune the wavelength (Ciurczak et al., 2021; Crocombe, 2018). In MIRS, Fourier transform IR (FT-IR) spectrometers are the gold-standard, as the Michelson interferometer allows for higher optical throughput compared to dispersive instruments, which is a crucial advantage when using low-power radiation sources like thermal emitters (Griffiths & De Haseth, 2007). Such spectrometers are also available in the NIR region, and their usage is increasing for the analysis of agricultural commodities. The differences in instrumentation used for NIRS and MIRS analysis of agricultural samples are not only caused by different sources, detectors, and wavelength discrimination elements required for spectrometer design, but mainly due to the sampling interfaces employed for recording either a MIR or NIR spectrum. The differences in probing the sample via NIRS or MIRS are a consequence of the difference in the absorbance coefficients of overtones and fundamental vibrations, as described previously. Generally, IR spectra are plotted as an absorbance (A), transmittance (T), or reflectance (R) spectrum. For recording the T of a sample, two measurements are required (Equation 1). Initially, the intensity of the

incident beam (I_0) is recorded, subsequently the beam intensity transmitted through the sample (I) is measured. A is the negative decadic logarithm of T and directly proportional to the concentration (c) of an absorber, the sample thickness or optical path length (d), and the molar decadic absorption coefficient (ε) of the analyte and hence is convenient for quantitative investigations. This relationship is commonly known as Beer's law (Griffiths & De Haseth, 2007; Workmann, 2012).

$$T = \frac{I}{I_0}; A = \log_{10}\left(\frac{I_0}{I}\right) = -\log_{10}(T) = \varepsilon c d; \quad (1)$$

$$R = \frac{I_s}{I_{Ref}}; A = \log_{10}\left(\frac{I_{RR}}{I_{RS}}\right) = -\log_{10}(R) = \varepsilon c d; \quad (2)$$

The R of a sample is recorded in a similar fashion as the T of a sample (compare Equations (1) and (2)) (Griffiths & De Haseth, 2007). The literature on NIRS for the analysis of mycotoxins in crops predominately reports spectra recorded in diffuse reflection (DR) mode (Section 3). When recording a DR spectrum, the incident radiation is not only attenuated due to absorption but also by scattering effects caused by the sample surface or scattering induced by powdered samples. Consequently, when recording a DR spectrum not only the absorption (i.e., chemical information) of the sample but also its scattering properties (i.e., physical information) are probed (Griffiths & Dahm, 2007). Beert's law (Equations (1) and (2)) does not account for scattering effects and therefore DR spectra can only be used within a narrow range for quantitative analysis (Griffiths & De Haseth, 2007). Even if spectra of nonscattering samples are investigated for mycotoxin contamination at trace levels, Beert's law cannot be used to obtain the concentration of mycotoxins in crop samples, as the expected absorbance of mycotoxins at such levels is too small to be directly detectable with classic IR spectrometers. An in-depth discussion on the determination of mycotoxins with IR spectroscopy is provided in sections 3 and 4.

However, Beert's law can give an indication on the wider usage of NIRS for the analysis of agricultural commodities compared to MIRS. The adsorption coefficient (ε) of overtones is at least a magnitude lower than the ε of the fundamental vibration enabling significantly higher optical pathlengths (d) in NIRS compared to MIRS (Workmann, 2012). Therefore, sample handling for MIRS is relatively challenging, but more straightforward for NIRS. In fact, solid agricultural or food samples, as commonly investigated during mycotoxin analysis, can be considered opaque in the MIR region, whereas NIR radiation can penetrate samples relatively deep. The low absorbance coefficients of overtone bands facilitates the analysis of a large sample volume (up to 200 g) with NIRS, allowing for the analysis of intact kernels and their corresponding mycotoxin contamination levels (Börjesson et al., 2007; Dvořáček et al., 2012; Levasseur-Garcia & Kleiber, 2015; Ruan et al., 2002). This difference in absorbance coefficients between the NIR and MIR regions led to the development of different sampling techniques prominent in MIRS and NIRS as discussed in section 3.1.

As shown in Figure 1, NIR spectra (Figure 1a) and MIR spectra (Figure 1b) differ significantly. IR spectroscopy provides a wealth of information, which is often overwhelming for inexperienced operators. IR spectrum interpretation of biological samples, like grain is thus challenging, as information of the complete sample composition is obtained. When recording an MIR spectrum of a grain sample, absorption features linked to the major biomolecules (i.e., proteins, carbohydrates, lipids, nucleic acids) dominate the corresponding IR spectrum (Figure 1a). The absorption features linked to proteins are mainly found in the Amide I (1650 cm^{-1}) and Amide II (1550 cm^{-1}) regions. Lipids absorb between 3000 cm^{-1} and 2800 cm^{-1} (CH stretch) as well as at 1750 cm^{-1} (C = O). The absorbance between 1100 cm^{-1} and 1000 cm^{-1} is caused by C-O ring vibration of carbohydrates (Baker et al., 2014). In contrast to these well-separated absorption features clearly assignable to certain biomolecules (Figure 1b), the information found in the corresponding NIR spectra of the same grain sample is hard to interpret. For example, the broad band between 1450 and 1650 nm arises due to the contributions of the NH stretching vibration from protein and OH stretching vibrations of carbohydrates. The band around 1900 nm is caused by OH combinational vibrations and is classically used to evaluate the moisture content of agricultural samples, but also contains information linked to polysaccharides and protein as NH combinational vibrations are also found in this region (Williams et al., 2019; Workmann, 2012). The same difficulties are encountered when assigning bands within NIR spectra of agricultural samples (e.g., wheat) over the whole spectral region, as all major biomolecule groups feature CH and OH moieties. Distinct spectral difference between fungi infected and non-infected samples can be found in NIR and MIR spectra (Fernández-Ibañez et al., 2009; Kos et al., 2004). DR NIR spectra are commonly plotted as $\log(1/R)$ vs. wavelength (Figure 1a), R being the diffusely reflected intensity from the sample. Using $\log(1/R)$ is an attempt to transform the reflectance of a diffusely reflecting sample into an absorbance like quantity for quantitative analysis. The absorption of the sample increases from the lower to the higher wavelengths in the NIR spectrum, as the absorption coefficients increase as the order of the overtone decreases. The baseline offset typical for DR NIR spectra is caused by scattering effects and increases with the wavelength. Consequently, the NIR spectrum of a ground wheat

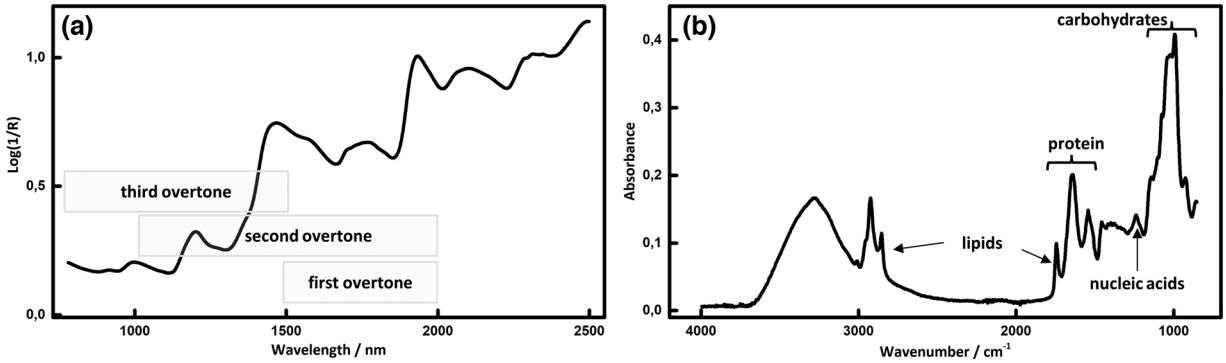

FIGURE 1 Near-infrared (NIR) and mid-infrared (MIR) spectrum of the same ground wheat sample. (a) Diffuse reflection NIR spectrum highlighting overtone region linked to bands of CH, OH, and NH vibrations and (b) Attenuated total reflection MIR spectrum with spectral regions assigned to certain biomolecules

sample shown in Figure 1a contains not only chemical information caused by the absorption of functional groups of certain biomolecules, but also physical information due to scattering effects caused by the powdered texture of the sample. The MIR spectrum shown in Figure 1b recorded using an attenuated total reflection (ATR) spectrometer on the other hand mainly contains chemical information, as ATR spectroscopy is not effected by classic scattering effects. Nevertheless, the particle size of a powdered sample influences the obtained absorbance, as the volume occupied in the evanescent field depends on the particle size (Milosevic, 2012). Therefore, smaller particles should not only increase the spectral reproducibility as shown by Kos et al. (2007), but also the absorbance obtained by ATR spectroscopy.

3 | LITERATURE ON MYCOTOXIN ANALYSIS USING IR SPECTROSCOPY

Mycotoxins are a diverse group of contaminates with unique IR spectroscopic fingerprints, significantly different to the absorbance characteristics of the major constituents of a grain sample, that is, carbohydrates, proteins, lipids, and nucleic acids. However, the low concentration of mycotoxins does not allow for their direct detection via IR spectroscopy in agricultural samples (Levasseur-Garcia, 2012). The absorbance for mycotoxins in these samples can be expected to be in the order of 10^{-6}, whereas the absorbance of the major constitutes of the sample is between 0.1 and 1 (Figure 1). The detection of such low mycotoxin signals exceeds the noise floor achievable with routine spectrometers. Moreover, the absorbance characteristics of the major constitutes and of mycotoxins are overlapping, obscuring important spectral information. Nevertheless, numerous publications have showcased the use of IR spectroscopy for monitoring mycotoxin contamination in different crops. These publications are based on the fact that IR spectroscopy is a strong tool when assessing sample composition, for example, carbohydrate and protein content, among other parameters (Williams et al., 2019). As mycotoxigenic fungi grow on the sample, the metabolization of the sample into fungal biomass results in changes of the major constituents (Boyacioğlu & Hettiarachchy, 1995). The alteration and change to sample composition induced by fungal infestation can be tracked by IR spectroscopy (Fernández-Ibañez et al., 2009; Kos et al., 2004). Therefore, mycotoxin contamination can be indirectly analyzed using IR spectroscopic methods (Levasseur-Garcia, 2012). To link fungi-induced changes in sample composition to the amount of mycotoxin present in the sample, external reference analysis, for example, LC-MS/MS, is essential. The obtained reference values and the IR spectra are then analyzed using chemometric methods with the aim of developing models for the prediction of mycotoxin contamination in unknown samples. The challenges of implementing such a model are discussed later (Section 4). After such models are established, the benefits of IR spectroscopy outperform established methods for rapid mycotoxin determination (i.e., immunological assays), in respect to device operation, the usage of consumables, and sample throughput.

The first attempt to detect mycotoxigenic fungal contamination in corn has been reported by Greene et al. (1992) and was conducted using photoacoustic (PA) and DR MIRS. Since then, MIRS has been employed for the analysis of fungal infestation and mycotoxin contamination in ground samples mainly by ATR, but also using PA and DR MIRS (Table 1). In parallel, NIRS has been used to predict contamination with mycotoxigenic fungi and mycotoxins in different agricultural commodities probing the sample using NIR diffuse transmission (NIT) or DR NIRS (Table 2). Some comparative studies on the differences between NIRS and MIRS for the

TABLE 1 Mid-IR (MIR) literature for mycotoxin analysis

Commodity	Target contaminants	Spectral acquisition modes	Reference
Corn & corn oil	Fungal infection, DON	PA, ATR, DR	(Fu et al., 2014; Gordon et al., 1997; Gordon et al., 1998; Greene et al., 1992; Kos et al., 2004; Kos et al., 2001; Kos et al., 2002, 2003; Kos et al., 2007; Kos et al., 2016; Öner et al., 2019; Sieger et al., 2017; Wheeler et al., 1993)
Peanut & peanut oil	Fungal infection, AFLA B_1, B_2, G_1, G_2	ATR, PA	(Kos et al., 2016; Mirghani et al., 2001; Sieger et al., 2017; Yang et al., 2018)
Wheat	DON	ATR	(Abramovic et al., 2007)

Abbreviation: DON = deoxynivalenol, AFLA = aflatoxin, PA = photoacoustic, ATR = attenuated total reflection, DR = diffuse reflection.

TABLE 2 Near-IR (NIR) literature for mycotoxin analysis

Commodity	Target contaminants	Spectral acquisition modes	Reference
Wheat	Fungal infection, DON, ergosterol	DR, NIT	(Beyer et al., 2010; De Girolamo et al., 2014; De Girolamo et al., 2009; Delwiche, 2003; Delwiche & Hareland, 2004; Dowell et al., 1999; Dvořáček et al., 2012; Peiris et al., 2017; Pettersson & Åberg, 2003; Rasch et al., 2010; Shen et al., 2019; Siuda et al., 2008; B. Zhang et al., 2021)
Corn	Ergosterol, DON, FUMs, AFLA B_1, ZEN	DR	(Berardo et al., 2005; Darnell et al., 2018; Della Riccia Giacomo, 2013; Falade et al., 2017; Fernández-Ibañez et al., 2009; Levasseur-Garcia et al., 2015; Levasseur-Garcia & Kleiber, 2015; Shen et al., 2022; Tao, Yao, Zhu, et al., 2019; Tyska et al., 2021)
Barley	Ergosterol, DON	DR, NIT	(Börjesson et al., 2007; Caramês et al., 2020; Fernández-Ibañez et al., 2009; Ruan et al., 2002)
Rice	Fungal infection, AFLA B_1	DR	(Dachoupakan Sirisomboon et al., 2013; Dachoupakan Sirisomboon et al., 2019; Putthang et al., 2019; Qiang et al., 2014)
Oats	DON	DR	(Tekle et al., 2013)
Peanut	Fungal infection, AFLA B_1	DR	(Li et al., 2019; Tao, Yao, Hruska, et al., 2019; S. Zhang et al., 2021)

Abbreviations: DON = deoxynivalenol, FUM = fumonisin, AFLA = aflatoxin, ZEN = Zearalenone, DR = diffuse reflection, NIT = near infrared diffuse transmission.

analysis of mycotoxins have been also reported (Table 3). In addition to NIRS and MIRS, mainly used for the analysis of bulk samples, single kernel NIRS (SKNIR) and HSI have also been used for mycotoxin determination focusing on the analysis of individual kernels (Tables 4 and 5, respectively). Besides classic agricultural commodities as listed in Tables 1–5 MIRS, NIRS and HSI have also been used to analyze raisins (Galvis-Sánchez et al., 2007; Heperkan & Gökmen, 2016), chili powder (Tripathi & Mishra, 2009), almonds (Liang et al., 2015; Mishra et al., 2022), pistachios (Kheiralipour et al., 2016; Wu & Xu, 2019), garlic (Liang et al., 2015), figs (Durmuş et al., 2017), green coffee (Taradolsirithitikul et al., 2017), milk (Jaiswal et al., 2018), green tea (Cao et al., 2020), corn silage (Ghilardelli

TABLE 3 Studies comparing mid infrared spectroscopy (MIRS) and near infrared spectroscopy (NIRS) for mycotoxin analysis

Commodity	Target contaminants	Spectroscopic techniques	Reference
Corn	AFLAs	MIRS, NIRS, Raman	(Lee et al., 2015)
Rice	AFLAs	MIRS, NIRS	(Shen et al., 2018)
Wheat	DON, OTA	MIRS, NIRS	(De Girolamo, Cervellieri, et al., 2019; De Girolamo, von Holst, et al., 2019)
Barley	Ergot alkaloids	MIRS, NIRS	(Shi et al., 2019)

Abbreviations: AFLA = aflatoxin, DON = deoxynivalenol, OTA = ochratoxin A.

TABLE 4 Literature on single kernel NIR-spectroscopy

Commodity	Target contaminants	Spectral acquisition modes	Reference
Corn	Fungal infection, AFLA, FUM	DR, NIT	(Chavez et al., 2022; Cheng et al., 2019; Dowell et al., 2002; Pearson et al., 2004; Stasiewicz et al., 2017; Tallada et al., 2011)
Wheat	DON, NIV	DR	(da Silva et al., 2019; Delwiche et al., 2005; Dowell et al., 1999; Jin et al., 2014; Kautzman, Wickstrom, Hogan, et al., 2015; Kautzman, Wickstrom, & Scott, 2015; Lim et al., 2018; Peiris et al., 2016; Saito et al., 2009)
Barley	Fungal infection	DR	(Lim et al., 2018; Lim et al., 2017)

Abbreviations: AFLA = aflatoxin, FUM = fumonisin, DON = deoxynivalenol, NIV = nivalenol, DR = diffuse reflection, NIT = near infrared diffuse transmission.

TABLE 5 Literature on hyperspectral imaging (HSI) for mycotoxin analysis

Commodity	Target contaminants	Reference
Corn	Fungal infection, FUM, AFLA B_1	(Chu et al., 2020; Chu et al., 2017; Del Fiore et al., 2010; Firrao et al., 2010; Kandpal et al., 2015; Kimuli, Wang, Jiang, et al., 2018; Kimuli, Wang, Lawrence, et al., 2018; Lu et al., 2022; Parrag et al., 2020; Tao et al., 2020; Wang et al., 2014; Wang, Heitschmidt, et al., 2015; Wang, Lawrence, et al., 2015; Wang, Ni, et al., 2015; Williams et al., 2010; Williams et al., 2012; Yang et al., 2020; Zhao et al., 2017; Zhu et al., 2016)
Wheat	Fungal infection, DON, OTA, ergosterol	(Alisaac et al., 2019; Barbedo et al., 2017; Bauriegel et al., 2011; Berman et al., 2007; Dammer et al., 2011; Delwiche et al., 2010; Delwiche et al., 2019; Femenias, Bainotti, et al., 2021; Femenias, Gatius, et al., 2021; Liang et al., 2020; Nadimi et al., 2021; Ropelewska & Zapotoczny, 2018; Senthilkumar et al., 2016; Shao et al., 2020; Shi et al., 2020; Singh et al., 2012; Singh et al., 2007; Zhao et al., 2020)
Rice	Fungal infection	(Siripatrawan & Makino, 2015; Wu et al., 2020)
Oats	DON	(Tekle et al., 2015)
Peanuts	Fungal contamination	(He et al., 2021; Jiang et al., 2016; Qi et al., 2019; Qiao et al., 2017)
Barley	DON	(Su et al., 2021)

Abbreviations: FUM = fumonisin, AFLA = aflatoxin, DON = deoxynivalenol, OTA = ochratoxin A.

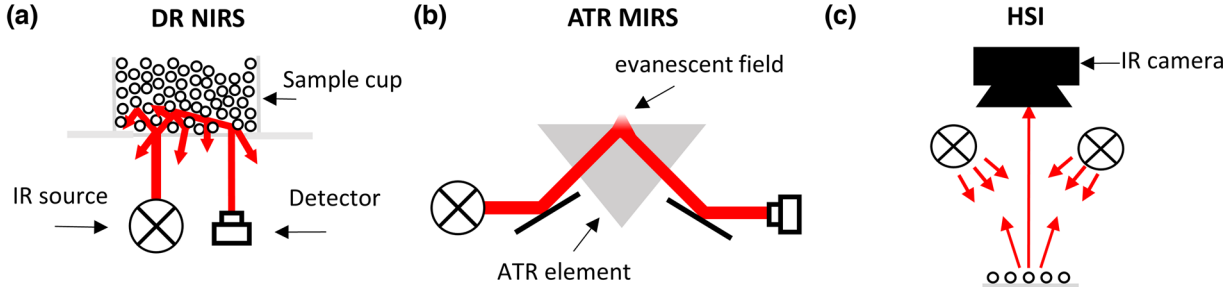

FIGURE 2 Typical settings for mycotoxin screening in near infrared spectroscopy (NIRS), mid infrared spectroscopy (MIRS), and hyperspectral imaging (HSI). Principle of (a) diffuse reflection (DR) NIRS, (b) attenuated total reflection (ATR) MIRS and (c) HSI

et al., 2022), and bio-ethanol byproducts (Tyska et al., 2022) regarding their contamination with mycotoxins or mycotoxigenic fungi infestation.

3.1 | IR spectroscopic techniques for mycotoxin screening

NIRS and MIRS are usually used to predict the mycotoxin contamination in a bulk sample. NIRS has been used more frequently compared to MIRS for the prediction of mycotoxins in bulk grain samples (compare Tables 1 and 2). This might be linked to the widespread use of NIRS for analyzing agricultural commodities facilitated by the ease of sample handling typical for NIRS. The dominance of NIRS and MIRS for bulk sample analysis is linked to the fact that HSI and SKNIR are used for the identification of fungal contamination on a single kernel level. Thus, HSI as well as SKNIR are more suitable for sorting applications to reduce the bulk mycotoxin concentration by removing single kernels highly infested with fungi, instead of obtaining bulk contamination levels.

As shown in Figure 2a, in a DR NIRS setting, the IR source and the detector are placed on the same side of the sample, typically underneath. A sample cup with an NIR transparent material is used to place the sample into the spectrometer. DR NIRS and ATR MIRS spectral acquisition allows for high sample throughput, as sample handling times of 2 min per sample are possible. Established rapid mycotoxin screening techniques such as immunoassays are significantly slower than 2 min (Bueno et al., 2015). The actual measurement time in NIRS is only a few seconds per sample, loading and cleaning of the sample cup consumes most of the user's time. In ATR spectroscopy, spectral acquisition takes longer compared to NIRS but cleaning the ATR element between samples can be performed within a few seconds, also enabling a sample throughput time of 2 min. Modern DR NIR spectrometers perform subsampling by rotating the sample cup, afterward averaging the recorded spectra. Due to the ease of sample handling typical for DR NIRS, NIR spectrometers for laboratory routine analysis of solid agricultural samples are predominantly operating in this mode. DR can also be used for MIRS. Due to the relative low intensity of thermal emitters and the strong absorption coefficients of fundamental vibrations, only a small amount of the sample can be analyzed at once, and moreover dilution in a nonabsorbing substance such as KBr is required (Griffiths & De Haseth, 2007). Therefore, DR is nowadays relatively unpopular in MIRS compared to its widespread use in NIRS. However, DR has been used for the analysis of mycotoxins in both NIRS and MIRS (Tables 1–3). In MIRS, ATR has evolved into the most popular sampling technique (Table 1). Similar to DR in NIRS, the extensive use of ATR in MIRS is linked to its robustness and ease of sample handling, simplifying MIR spectrum acquisition. In ATR MIRS, IR radiation is guided from the source through the ATR element toward the detector (Figure 2b). The ground crop sample is simply placed on top of the ATR element and probed by the so-called evanescent field. This field forming at the interface between the ATR element and the sample penetrates the sample commonly around 2 μm (Griffiths & De Haseth, 2007). ATR spectroscopy is especially useful for highly absorbing samples, like aqueous solutions or biological samples. As with such samples, path lengths of a few micrometers are required leading to cumbersome sample handling in other MIR spectroscopic settings (i.e., clogging of transmission cells) (Schwaighofer & Lendl, 2020). Solid samples like ground cereals, which are possibly contaminated with mycotoxins, need to be thoroughly pressed against the sampling interface to ensure good contact between the sample and ATR element. Insufficient contact leads to significant experimental errors. ATR elements in routine MIR spectrometer are relatively small with a size of approx. 2–3 mm in width, length, or diameter. This is useful for substance identification or in life-science applications, where precious samples are being investigated and only a very small amount of sample is available for analysis. However, when analyzing mycotoxin contamination in agricultural samples, the small size of the ATR element

is not necessarily advantageous, as the small sampling interface makes grinding of the sample and subsampling unavoidable (Kos et al., 2003, 2004). Probing bulk samples for mycotoxin analysis is in general a challenge and based on dedicated sampling plans including, mixing and milling steps to ensure sample homogeneity (Whitaker & Slate, 2012). To comply with official regulations, sample sizes of up to 30 kg need to first be obtained (Krska et al., 2008). It is obvious that ensuring the representativity of a small test sample of only a few mg (as needed for ATR spectroscopy) is quite a challenge and requires extremely careful homogenization. To counteract the introduced sampling error, subsampling is commonly performed when using ATR MIRS for mycotoxin analysis (De Girolamo, von Holst, et al., 2019; Kos et al., 2002, 2003). Kos et al. showcased that by including a sieving step, the spectral reproducibility of ATR measurements can be significantly improved and that the particle size indirectly scales the spectral reproducibility. They reported that sieving fractions with a size of 100–250 μm are well suited for ATR MIRS when screening for mycotoxin contamination (Kos et al., 2007). Such sample-handling steps like milling and sieving partly counteract the rapidness of IR spectroscopic approaches. NIRS, on the other hand, allows for the analysis of a larger sample volume at the same time. The amount of sample investigated by DR NIRS for mycotoxin contamination ranges from 30 g of ground to 200 g of intact grain sample (De Girolamo, von Holst, et al., 2019; Levasseur-Garcia & Kleiber, 2015). The blurred information obtained by NIR spectra comes with the advantage of probing a larger test sample. In contrast to classic spectroscopy, in HSI, spectra at specific spatial coordinates are recorded resulting in complex data cubes (Boldrini et al., 2012). For mycotoxin determination in agricultural samples Vis/NIR HSI has been widely exploited (Femenias et al., 2020). This is linked to the fact that imaging systems in the Vis/NIR spectral region are relatively cheap and fast, compared to other spectral regions like the MIR. Vis/NIR imaging systems have proven their applicability in process analytical technology and are therefore a promising candidate for the online screening for mycotoxin contamination. For mycotoxin determination, the NIR imaging system and the radiation source are placed above the sample (Figure 1c). Typically, intact kernels are analyzed using HSI and are commonly arranged separately to enable analysis of individual kernels (Chu et al., 2020; Delwiche et al., 2019).

3.2 | Differences between NIRS and MIRS for mycotoxin screening

MIR spectra are easier to interpret compared to NIR spectra (see Figure 1). Therefore, it might be expected that MIRS is superior to NIRS for the prediction of mycotoxin contamination. The literature comparing NIRS and MIRS (Table 3), however, reports inconsistent results. Lee et al. (2015) were the first to compare NIRS, MIRS, and Raman spectroscopy. They found that MIRS as well as Raman spectroscopy performs better than NIRS. Shen et al. on the other hand reported that NIRS is slightly superior compared to MIRS (Shen et al., 2018). In the work of De Girolamo et al. it was reported that NIRS is superior for DON determination, whereas for ochratoxin A (OTA), MIRS performs slightly better (De Girolamo, Cervellieri, et al., 2019; De Girolamo, von Holst, et al., 2019). Shi et al. aimed at the development of models to predict ergot alkaloid contamination combing LC-MS/MS with NIRS and MIRS. They found that neither NIRS nor MIRS can be used to obtain a usable model (Shi et al., 2019). The findings by Lee et al. may indicate that probing fundamental vibrations is superior to analyzing overtones of certain vibrations. However, the publications of De Girolamo et al. underline that this drawback of NIRS might be negated by probing a higher amount of sample with DR NIRS. This is especially true when ATR spectroscopy is compared with DR NIRS. In ATR spectroscopy, usually a tiny amount of sample is measured (<0.5 g), whereas De Girolamo reported that 30 g of sample was used for NIRS (De Girolamo, Cervellieri, et al., 2019; De Girolamo, von Holst, et al., 2019). This drawback is further described by Lee et al. (2015), who used DR MIRS as they found DR to have a higher reproducibility than ATR. Previously, Kos et al. (2004) reported the opposite that ATR is superior to DR MIRS regarding its spectral reproducibility. In a series of publications, Kos et al. optimized an ATR-based workflow including sieving the ground sample to evaluate the influence of the grain size of the powdered sample. They showed that ATR MIRS can be a strong tool for developing models to predict mycotoxin content in different matrices (Kos et al., 2004, 2001, 2002, 2003, 2007, 2016). In this context, it should be noted that Abramovic et al. (2007) was able to omit this sieving step, by simply using a larger multibounce ATR element. They linked the higher spectral reproducibility to the fact that by using a larger ATR element, a higher amount of sample is probed, and the influence of particle size is reduced. Moreover, they also reported that DR is only slightly inferior than ATR. These inconsistent reports on DR MIRS for mycotoxin analysis from Lee et al., Kos et al. and Abramovic et al. underline the technical difficulties encountered when recording a DR MIR spectrum, which is well known in the MIR community besides being explicitly discussed by Kos et al. (2004). In general, obtaining reproducible MIR spectra of powdered samples is a challenge. DR MIRS probes more sample compared to ATR, hence facilitating a higher representativeness of a DR

spectrum, possibly reducing systematic errors. However, the challenges in sample handling and the thereby introduced error with DR MIRS negates this advantage. An interesting approach possibly counteracting such problems was reported by Sieger et al. (2017) in their publication; they used extracts of corn and peanut samples to establish models for discrimination at regulatory limits. They used a spectrometer relying on an extended tunable quantum cascade laser (EC-QCL) combined with a gallium arsenide waveguide. They employed extracts of the investigated crops, which is a unique approach in the respective mycotoxin literature and might help omit experimental errors introduced when measuring ground samples directly.

3.3 | NIRS for screening of mycotoxin contamination in bulk samples

NIRS for the determination of mycotoxins was initially used for single kernel analysis (Delwiche, 2003; Dowell et al., 1999). Analysis with NIRS of bulk grain regarding its mycotoxin contamination was first demonstrated for DON in barley using a DR NIR spectrometer (Ruan et al., 2002). Shortly afterward, usage of NIT for the determination of DON in wheat was reported (Pettersson & Åberg, 2003). These initial publications already impressively showed the promising character of NIRS for bulk grain analysis. NIT of whole kernels is routinely performed by grain traders to evaluate grain quality (Williams et al., 2019). Such an approach would have great potential for mycotoxin analysis, as moisture, protein, and mycotoxin content may be assessed at once during grain delivery. NIT devices operate in a narrow spectral range between 670 and 1100 nm, enabling the required optical pathlength so that intact kernels can pass through the device. Börjesson et al. (2007) highlighted that the spectral information obtained by NIT devices is sufficient for mycotoxin screening and can compete with benchtop NIR spectrometers using a broader spectral range. After this initial work of Pettersson et al. and Börjesson et al. using NIT, the scientific community predominantly employed DR NIRS. While whole kernel analysis is easily doable using NIRS, the sample under investigation is often milled in routine analytical and immunoanalytical procedures to increase the sample homogeneity (Whitaker & Slate, 2012). Thus, many scientists milled the sample before recording a DR NIR spectrum. De Girolamo et al. predicted the DON content in ground common and durum wheat, after exploring a sieving step using FT-NIR. They found that even though there are spectral differences between the different wheat species, their model could predict the DON content below the levels set by the European Commission (De Girolamo et al., 2009). Several other authors employed grinding of the sample when building NIR spectroscopic models to meet regulatory requirements (Caramês et al., 2020; De Girolamo et al., 2014, 2009; Della Riccia Giacomo, 2013; Tyska et al., 2021). Levasseur-Garcia et al. showed that mycotoxin prediction models can also be developed without grinding, while also ensuring compliance to regulations. Thus, simplistic sample handling can be achieved when relying on a benchtop DR NIR spectrometer (Levasseur-Garcia et al., 2015; Levasseur-Garcia & Kleiber, 2015). Recently, the use of a portable NIR spectrometer for analysis of FUM in corn has been demonstrated (Shen et al., 2022). The reported method still relies on using a laboratory grade mill for sample grinding and thus, hindering in-field or on-site applicability.

3.4 | HSI and SKNIR for mycotoxin screening on a single kernel level

In recent years, HSI has been increasingly exploited for mycotoxin analysis in agricultural samples (Femenias et al., 2020). NIR-HSI allows for the analysis of several kernels at once; in the literature (listed in Table 5) kernels are often analyzed after prearrangement. In an early study, HSI was used to discriminate between sound and stained (decolorized and colorized) wheat kernels (Berman et al., 2007). It has been reported that color imaging might be superior to Vis-HSI and interestingly fungal infected kernels could be identified via HSI by using just one wavelength thus, facilitating low image acquisition times (Singh et al., 2007). In 2010, Del Fiore et al. (2010) reported the possibility to discriminate between healthy and fungi infected corn kernels using HSI. However, they also used the visible region to identify contaminated kernels. Several other HSI papers listed in Table 5 do not often rely on the IR region, as the employed HSI systems normally operate between 400 and 1000 nm. Analysis is often based on the information found within the visible region. This is linked to the fact that fungal infestation often effects coloration (Berman et al., 2007). The first HSI paper solely relying on the NIR region for quality assessment of wheat was published by Delwiche et al. (2010), who showcased that healthy and *Fusarium* infected wheat kernels can be straightforwardly discriminated, by relying on only two pairs of wavenumbers. Williams et al. compared two NIR HSI systems operating from 960 nm to 1662 and 1000 to 2498 nm. They concluded that the latter instrument featuring a broader spectral range is advantageous for discriminative analysis (Williams et al., 2010). Subsequently, they also reported a model that could predict the degree of fungal infestation (Williams et al., 2012). Bauriegel et al. (2011) impressively showcased the potential of HSI by reporting the possibility

to discriminate areas infected by *Fusarium* from healthy areas directly in the intact ear. In-field applications have also been examined (Dammer et al., 2011). In a series of publications, Wang et al. explored the possibilities of using NIR-HSI for the detection of AFLAs in corn kernels (Chu et al., 2017; Wang et al., 2014; Wang, Lawrence, et al., 2015; Wang, Ni, et al., 2015). HSI is predominately used to measure intact kernels; the technique has been also utilized for the analysis of milled grain (Femenias, Gatius, et al., 2021; Firrao et al., 2010; Parrag et al., 2020; Zhao et al., 2020). Parrag et al. reported the use of HSI to set-up models capable of either quantitative or discriminant analysis using milled samples. Femenias et al. compared the influence of sample grinding on the influence of models for mycotoxin determination with the HSI data of unground samples. They found that models based on intact kernels performed best. HSI can not only be used to analyze several kernels simultaneously but also enables the investigation of several samples at once (Shao et al., 2020). Therefore, HSI holds additional potential for increasing sample throughput.

The usability of SKNIR spectroscopy for mycotoxin contamination reduction has been summarized by Chavez et al. (2020). Single kernel analysis is a tedious task, as one spectrum per kernel needs to be recorded, but has great potential for grain sorting to reduce mycotoxin contamination (Delwiche & Hareland, 2004). Pearson et al. (2004) showcased that by single-kernel near infrared spectroscopy (SKNIR) with a commercially available device, the AFLA and fumonisin (FUM) contamination of the bulk corn sample could be reduced significantly. Several other authors used SKNIR for mycotoxin reduction and prediction (Saito et al., 2009; Tao, Yao, Zhu, et al., 2019) to assist breeding programs (da Silva et al., 2019; Jin et al., 2014; Peiris et al., 2010), to use downgraded lots in feeding trials (Kautzman, Wickstrom, Hogan, et al., 2015) or to salvage high-quality grain from downgraded lots (Kautzman, Wickstrom, & Scott, 2015). Stasiewicz et al. (2017) developed a compact, low-cost optical sorter employed for the reduction of AFLAs and FUMs in Kenyan corn. Their system was further refined and used to screen for AFLAs and FUMs on a single corn kernel level, underlining the influence of single, highly contaminated kernels on the bulk mycotoxin content of a sample (Chavez et al., 2022; Cheng et al., 2019).

When comparing both techniques for sorting tasks, SKNIR appears to be more fitting for in-field implementation as the use of commercial devices and custom-made, cost-efficient devices has been reported (Delwiche et al., 2005; Stasiewicz et al., 2017). By relying on simple data analysis schemes, real-time identification of infected kernels was possible (Chavez et al., 2022; Delwiche et al., 2005; Pearson et al., 2004; Stasiewicz et al., 2017). HSI methods for single kernel analysis on the other hand often rely on well-separated previously aligned kernels. Hence, an additional sample-handling step is required, further complicating implementation. In SKNIR, such pre-alignment steps can be partly omitted as the device itself facilitates kernel separation. In HSI usually a vast amount of data is recorded per image, complicating data analysis. Identification of fungi-infected kernels is, however, doable by only relying on one or two wavelengths, thereby significantly simplifying data analysis, and thus increasing imaging or sorting speed (Delwiche & Gaines, 2005; Delwiche et al., 2010; Singh et al., 2007). However, the vast amount of knowledge from other sorting applications relying on HSI could be beneficial for the sorting of contaminated grain kernels.

As shown in Tables 1–5, there is a wide range of literature using different types of IR spectroscopy to analyze agricultural commodities for mycotoxin or mycotoxigenic fungal contamination, including NIRS, MIRS, SKNIR, and HSI. Only few of those publications showcased that IR spectroscopic methods might be usable for the clearance of bulk samples at regulatory limits and will be discussed later (Section 5, Table 6).

4 | IMPLEMENTATION OF IR SPECTROSCOPY FOR THE DETERMINATION OF MYCOTOXINS

Mycotoxin contamination is indirectly determined using IR spectroscopic methods by measuring sample changes induced by fungal growth (see Section 3). The IR spectrum and the mycotoxin level, obtained by reference analysis, are used in chemometric data analysis methods. The developed models aim to either discriminate between safe and contaminated samples (or kernels in SKNIR or HSI) at a certain threshold or to quantitatively predict mycotoxin content.

In Figure 3, the workflow for the implementation of IR spectroscopy for mycotoxin analysis is illustrated. As indicated in Figure 3, four prerequisites need to be met to implement IR spectroscopy for mycotoxin screening: (i) fitting sample sets, (ii) an IR spectrometer, (iii) accurate reference analysis, and (iv) data analysis (i.e., chemometric methods) for model development.

4.1 | Sample collection for the development of an IR calibration

Prediction of mycotoxins at trace levels in crops via IR spectroscopy is conducted in an indirect manner, by linking changes in sample composition due to fungal infestation with the concentration of the contaminant obtained by external reference analysis alongside chemometric

TABLE 6 Literature exploring the applicability of infrared (IR) spectroscopy for mycotoxin screening in bulk samples at guideline values

Commodity	Mycotoxin	Spectroscopic techniques	Model type, sample size, accuracy, prediction error, concentration range	Category	Reference
Corn	DON	ATR MIRS	Discriminative and quantitative; $n = 14$, 100%, RMSECV = 494.5 $\mu g\ kg^{-1}$, 310–2596 $\mu g\ kg^{-1}$	B	(Kos et al., 2003)
Corn	DON	DR & ATR MIRS	Discriminative and quantitative; $n = 21$; 100%, RMSECV = 438.7 $\mu g\ kg^{-1}$, 310–2596 μg kg-1	B	(Kos et al., 2004)
Corn	DON	ATR MIRS	Discriminative; $n = 14$, 100%, 310–2596 $\mu g\ kg^{-1}$	B	(Kos et al., 2007)
Wheat (durum + winter)	DON	DR NIRS	Discriminative and quantitative; $n = 262$; 69%, RMSECV = 379 $\mu g\ kg^{-1}$, 0–3000 $\mu g\ kg^{-1}$	B	(De Girolamo et al., 2009)
Corn	FUMs	DR NIRS	Quantitative; $n = 143$; RMSEP = 0.917 mg kg^{-1}, 0.417–11.845 mg kg^{-1}	B	(Gaspardo et al., 2012)
Corn	FUMs	DR NIRS	Quantitative; $n = 133$; RMSEP = 0.890 mg kg^{-1}, 0.357–11.845 mg kg^{-1}	B	(Della Riccia Giacomo, 2013)
Wheat	DON	DR NIRS	Discriminative and quantitative, $n = 464$; 90%; RMSEP = 1977 $\mu g\ kg^{-1}$, < 50–16000 $\mu g\ kg^{-1}$	B	(De Girolamo et al., 2014)
Peanut	AFLA	ATR MIRS	Discriminative; $n = 164$; 98.5% < 20 - > 1200 $\mu g\ kg^{-1}$	B	(Kaya-Celiker et al., 2014)
Corn	FUM	DR NIRS	Discriminative; $n = 117$; 82%, 60–9850 $\mu g\ kg^{-1}$	B	(Levasseur-Garcia et al., 2015)
Corn	DON, FUMs	DR NIRS	Discriminative; DON $n = 381$ 72- 77%, 0–9530 $\mu g\ kg^{-1}$; FUM $n = 511$ 70–72%, 24 - 5509 $\mu g\ kg^{-1}$	B	(Levasseur-Garcia & Kleiber, 2015)
Corn, Peanut	DON, AFLA B_1	ATR MIRS	Discriminative; Corn, DON n = 110, 79%, < 1.5–50160 $\mu g\ kg^{-1}$; Peanut AFLA B_1 $n = 92$; 77%, < 0.24–10624 $\mu g\ kg^{-1}$;	B	(Kos et al., 2016)
Corn	DON	ATR MIRS	Discriminative; $n = 183$; 92%, < 1.5–50160 $\mu g\ kg^{-1}$	A	(Öner et al., 2019)
Wheat	OTA	DR NIRS, ATR MIRS	Discriminative; $n = 255$; 94%, < 0.15 to 40 $\mu g\ kg^{-1}$	A	(De Girolamo, von Holst, et al., 2019)
Barley	DON	DR NIRS	Quantitative and Discriminative; $n = 60$; 90.4%; RMSEP = 160.76 $\mu g\ kg^{-1}$, 64 −2131 $\mu g\ kg^{-1}$	A	(Caramês et al., 2020)
Wheat	DON	HSI	Quantitative and Discriminative; $n = 270$; 85,4% RMSEP = 1.17 mg kg^{-1},< 50- 3537 $\mu g\ kg^{-1}$	B	(Femenias, Gatius, et al., 2021)
Corn	FUMs, ZEN	DR NIRS	Quantitative; FUMs, $n = 236$; RMSEP = 659 $\mu g\ kg^{-1}$, 125–24200 $\mu g\ kg^{-1}$; ZEN, $n = 440$; RMSEP = 69.7 $\mu g\ kg^{-1}$, 20–884 $\mu g\ kg^{-1}$	A	(Tyska et al., 2021)
Wheat	DON	DR NIRS plus machine vision	Discriminative; $n = 190$, 93.55 %; 12–3755 $\mu g\ kg^{-1}$	A	(B. Zhang et al., 2021)

Abbreviations: DON = deoxynivalenol, FUM = fumonisin, AFLA = aflatoxin, OTA = ochratoxin A, ATR = attenuated total reflection, MIRS = mid infrared spectroscopy, DR = diffuse reflection, NIRS = near infrared spectroscopy, HSI = hyperspectral imaging, RMSECV = root mean square error of cross validation, RMSEP = root mean square error of prediction.

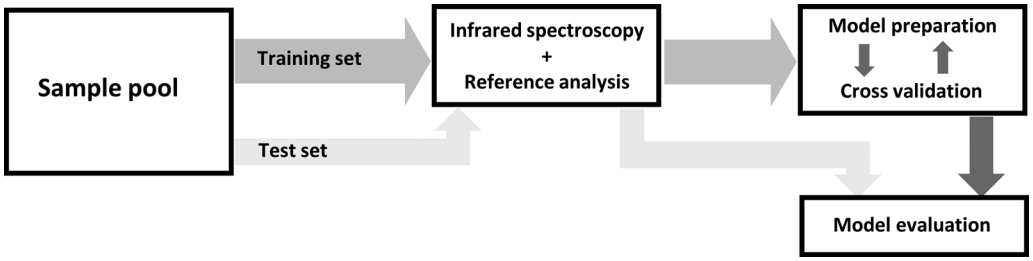

FIGURE 3 Diagram of the steps needed for developing and implementing models based on infrared spectroscopy for mycotoxin prediction

methods. To develop robust mycotoxin prediction models capable of predicting contamination in samples from different geographical regions and years including different genotypes in a routine analytical setting requires the collection of several hundred samples. (De Girolamo, von Holst, et al., 2019; Levasseur-Garcia & Kleiber, 2015; Öner et al., 2019; Tyska et al., 2021). This must be done to obtain the required, natural variance of the samples. Other more direct spectroscopic approaches like surface enhanced Raman spectroscopy (SERS) allow for simply spiking ground blank grain sample with the mycotoxin of interest (i.e., matrix matched calibration) or to simply use external calibration (Liu et al., 2020; Zhang et al., 2020). However, direct detection of mycotoxins is not feasible with IR spectroscopy due to the limited sensitivity and the overlapping absorption features of the major components of the sample (e.g., proteins, carbohydrates, and lipids) and thus, making classic calibration approaches not applicable. Therefore, naturally contaminated samples from different batches, years, genotypes, and geographical regions but from the same commodity must be collected and analyzed with IR spectroscopy alongside a reference method. The sample variance poses as challenge for model preparation based on IR spectroscopy, as agricultural products from different years, regions, or genotypes vary in their composition significantly as these differences, reflected by the IR spectra, have been used for food authentication or the rapid analysis of grain quality (Ciurczak et al., 2021; Danezis et al., 2016; Williams et al., 2019). Therefore, it must be ensured that the model is based on the variance of the samples due to fungal infestation, and not on the variance caused by seasonal and regional differences. This can be difficult as fungal infestation varies significantly in different regions (Khaneghah et al., 2018) and thus regional difference might correlate with mycotoxin levels. The information on origin, year, and batch is highly beneficial for model development and validation, as sample sets can be split along these differences into training and test sets or in folds for cross-validation (Figure 3). For example, a model based on samples from 1 year might be tested for its predictive performance using samples from another year. For exploratory studies, that is, when a certain spectroscopic method is tested for its applicability, a smaller naturally contaminated sample set (e.g., from one season) or samples inoculated with mycotoxigenic fungi either in the lab, greenhouse, or field might be used. Inoculation with fungi strains often selected for their ability to produce high amounts of mycotoxin might not actually reflect the natural occurrence of mycotoxigenic fungi. Information on the mycobiota found in the analyzed sample set might prove beneficial, as some plant pathogens attack crops (i.e., *Fusarium avenaceum*) but do not produce regulated compounds (Uhlig et al., 2007).

4.2 | Choosing the IR spectroscopic method

Choosing the right IR spectroscopic technique to obtain spectral data for model development depends on the intended application (bulk sample vs. single kernel analysis). For the determination of mycotoxin contamination in a bulk sample, milling is often used to increase homogeneity of the test sample (Whitaker & Slate, 2012). Such ground samples can be easily analyzed using either DR NIRS or ATR MIRS (De Girolamo, von Holst, et al., 2019). The ease of sample handling with DR NIRS outperforms even ATR MIRS, which is commonly known as a robust and straightforward technique in the MIR spectroscopic community. The additional and clearer information contained in a MIR spectrum might justify the slightly more complicated sample handling, depending on the application. For pasty samples like ground peanut (Kaya-Celiker et al., 2014; Kos et al., 2016), ATR spectroscopy presents itself as the method of choice, as routine ATR spectrometers are unmatched in their cleanability. The homogeneity of liquid samples like oils enables straightforward analysis via ATR spectroscopy; thus, the spectroscopic wealth of the MIR region can be easily exploited (Fu et al., 2014; Yang et al., 2018). Analysis of bulk samples regarding its mycotoxin contamination using intact kernels is doable using either NIT, DR NIRS, or HSI (Femenias, Gatius, et al., 2021; Pettersson & Åberg, 2003; Tyska et al., 2021). Additionally,

for removing fungi-infected kernels to reduce the mycotoxin concentration of a bulk sample, SKNIR or HSI imaging can be used (Chavez et al., 2020; Femenias et al., 2020).

4.3 | Reference analysis

The precision and accuracy of the reference method used to obtain the mycotoxin level influences the achievable performance of the developed mycotoxin prediction model. In the literature, a wide variety of reference methods has been reported to obtain the needed mycotoxin value of samples to be analyzed alongside IR spectra using chemometric methods. Visual inspection of samples by trained personnel over immunoanalytical methods (i.e., ELISAs) to sophisticated routine analytical techniques like LC-MS/MS have been reported for this purpose (Beyer et al., 2010; Kaya-Celiker et al., 2014; Tyska et al., 2021). The choice of the reference method predominantly depends on the aim of the developed model and on the financial resources, as mycotoxin analysis with routine analytical techniques is relatively expensive. For discriminative models used to distinguish between certain contamination levels, less complex, cost-effective analytical techniques (e.g., ELISAs, LFAs) can be employed for reference analysis. When aiming for quantitative prediction, validated routine analytical techniques should be favored. The initial higher cost for obtaining mycotoxin reference values with sophisticated reference methods is justified, as once the model is established, a vast amount of analyses profit from this initial investment. Moreover, accurate routine multi-toxin methods, such as LC-MS/MS usually provide information on a greater variety of mycotoxins and other secondary fungal metabolites potentially present in the studied samples (Sulyok et al., 2020). This additional information might also be beneficial for model development. The inferior precision of immunoanalytical methods, like ELISAs and LFAs, can be counteracted by measuring several aliquots of the same sample. It has been shown that by doing so, ELISAs can reach an analytical performance similar to routine analytical methods (Focker et al., 2019). The values obtained by the reference method should not be seen as absolutes, as each analytical method comes with a particular level of uncertainty. The influence of measurement uncertainty for the employed reference method often remains unconsidered during model development. Barbedo et al. (2017) reported that their HSI-NIR model could be significantly improved when measurement uncertainties of the employed reference methods were taken into consideration. By doing so, these authors show the importance of accurate and precise reference analysis for the development of mycotoxin prediction models relying on IR spectroscopy.

4.4 | Model development using chemometric methods

Chemometric methods combined with spectral pretreatment forms the basis for mycotoxin prediction model development. As shown in Figure 3, collected samples are ideally split into training and test sets. All samples are analyzed using IR spectroscopy and the chosen reference method. The training set (i.e., calibration set) is used for model development, the test set for evaluation of the developed model. The model generated using the training set is used to predict the mycotoxin content in the test set. The errors between these predicted values and the values obtained by reference analysis serve as a measure of the model performance. IR spectra are usually preprocessed before being subjected to chemometric analysis. These procedures such as multiplicative scatter correction (MSC) or derivatives aim to remove baseline drifts, spectral distortions due to scatter effects and to enhance spectral information (Agelet & Hurburgh Jr.,2010). The choice of spectral preprocessing procedures depends on the investigated sample and the spectrum acquisition mode. DR NIR spectra, for example, are treated differently than ATR MIR spectra. When building models based on HSI data specific image processing steps are often performed (e.g., removal of bad pixels), before preprocessing the spectra and chemometric analysis (Boldrini et al., 2012). A multitude of chemometric methods have been used for developing mycotoxin prediction models based on IR spectra and reference data. Partial least squares (PLS) is the dominantly used method within the literature (Tables 1–5), as this technique is well suited for handling highly collinear data (e.g., IR spectra) and can be used for quantitative prediction and classification approaches. Several publications explore wavelength selection or exclude certain spectral regions for model development; this approach seems to be especially useful when relying on HSI data (Delwiche et al., 2010; Delwiche et al., 2019; Singh et al., 2007). More complex algorithms like artificial neural networks (ANN) or support vector machine (SVM) have been also explored for mycotoxin model development among many other techniques (Öner et al., 2019; Ruan et al., 2002). For an in-depth introduction to the basics of chemometric techniques and their implementation, the reader is referred to the book by Varmuza and Filzmoser. A more detailed tutorial for model development based on NIR spectra is given by Agelet and Hurburgh (Agelet & Hurburgh Jr., 2010; Varmuza & Filzmoser, 2016).

The usability of the models based on IR spectra and developed by chemometric methods can be evaluated by the reported statistical terms. In discriminative models, the performance of the model is usually depicted as confusion matrix (Varmuza & Filzmoser, 2016). The classification

accuracy and other descriptive terms for discriminative models can be calculated based on this matrix and are reported as percentage.

Quantitative prediction models can be evaluated by several factors. The coefficient of determination (R^2) serves as a measure of the capability of the model to explain the dataset. The root mean square error (RMSE) reports the accuracy of quantitative models (Varmuza & Filzmoser, 2016; Williams et al., 2019). In NIRS-based mycotoxin prediction models, the ratio of prediction to deviation (RPD) is sometimes reported, which is the ratio of the standard deviation of a variable and the standard error of prediction of that variable (Shen et al., 2019; Tyska et al., 2021). This unitless value describes the ability of the model to predict the mycotoxin contamination in samples not included during model set up and is commonly used in NIRS (Williams et al., 2019).

Ideally, discriminative models should have a predictive accuracy of 100% (e.g., all samples are assigned to the correct class) and, for quantitative models, an RMSE of 0 would be desirable. Obviously, such values cannot be obtained in real-life scenarios as certain sources of errors cannot be avoided and some variance in datasets will always remain unexplained. Therefore, by evaluating the mycotoxin prediction models summarized in Table 6, several factors need to be considered to judge the readiness for implementation. The number and the variety of samples involved in model set up miggive an indication of the robustness of the reported model. Small numbers, or samples from just 1 year, region, or genotype often indicate overfitting; hence, prediction of mycotoxin levels in samples not used during model set-up is likely not to work. The reported accuracy of either quantitative or discriminative models should ideally be reported for an external test set (i.e., not involved in model set-up or model validation) and must be critically evaluated as described above.

5 | APPLICABILITY OF IR SPECTROSCOPY AT GUIDELINE VALUES

Several authors have explored the applicability of IR spectroscopy for mycotoxin screening at legal limits. Publications reporting models that are capable of either discriminating at a certain threshold or to quantitatively predict mycotoxin contamination at regulatory guideline values are summarized in Table 6.

NIRS and MIRS spectroscopy dominates the list of publications exploring the use of IR spectroscopy for rapid mycotoxin screening in bulk sample to meet regulatory requirements. Methods for the determination for the *Fusarium* toxin DON are most frequently reported (Table 6). This is linked to the fact that DON is among the most prevalent mycotoxins, thus causing significant health impacts and economic losses (Eskola et al., 2020; Khaneghah et al., 2018). Hence, DON poses as an important analyte for exploring the capabilities of IR spectroscopy. Contamination of other *Fusarium* toxins like ZEN and FUMs in corn has only yet been analyzed with NIRS (Table 6). Prediction of AFLA in peanut and corn, on the other hand, was exclusively analyzed with MIRS with respect to meeting regulatory limits. Determination of AFLA and OTA is in general more challenging compared to screening for *Fusarium* toxins, as those mycotoxins are highly toxic and hence the regulatory limits are several magnitudes lower than those of DON, ZEN, or FUMs (Eskola et al., 2020). In addition, the AFLA or OTA content of samples is more likely to be not heterogeneously distributed throughout the sample, as opposed to *Fusarium* toxins (Krska et al., 2008). Kaya-Celiker et al. (2014) reported that they could discriminate between peanuts meeting the regulatory requirements of AFLA contamination below 20 μg kg^{-1} and higher contaminated material using ATR MIRS. Kos et al. (2016) chose an AFLA threshold of 8 μg kg^{-1} for discrimination, underlining the capability of developing models relying on ATR MIRS. The predominant use of NIRS for the determination of *Fusarium* toxins and ATR MIRS for AFLA indicates that the marginal higher complexity of the latter technique might be justified. Thus, more challenging analytical problems can be approached by using the additional information contained in an MIR spectrum, compared to the corresponding NIR spectrum. Additionally, the relatively small ATR element facilitates easy clean-up compared to sample cups commonly used in NIRS. Therefore, ATR MIRS is especially useful for analyzing pasty samples like ground peanuts. Femenias, Gatius, et al. (2021) recently demonstrated that HSI imaging may be used to screen for DON in bulk samples at regulatory limits. Interestingly, they found that their method performed better using whole kernel samples, compared to ground samples. Their paper further underlines the promising character of HSI for online or at line implementation.

The publications listed in Table 6 exploring the use of IR spectroscopy to meet regulatory requirements report a wide range of accuracies for their developed models. Thus, the methods reported in those publications might not be directly implementable but demonstrate that after proper refinement, real-life usability is feasible. Other methods reported in Tables 1–5 often make use of a different, higher mycotoxin concentration or simple aim for the detection of fungal infestation. By using fitting or an extended sample set, these methods might be also adopted for mycotoxin determination at regulatory limits. Kos et al. (2003) were the first to show that MIRS can be used to discriminate blank and DON contaminated corn samples at a

threshold of 310 μg kg^{-1} with an accuracy of 100%, also exploring the development of a model for quantitative prediction of DON using PLS regression. A threshold of 310 μg kg^{-1} seems to be quite arbitrary, as other regulatory limits for DON in corn are higher. However, these initial results were already quite impressive. The developed models were based on a small and uniform sample set (n = 14, one genotype) therefore making overfitting very likely. A larger sample set (n = 262) was analyzed by De Girolamo and her co-workers for the prediction of DON in durum and common wheat of 32 different varieties collected over 4 years. They reported an RMSEP of 379 μg kg^{-1} for their quantitative model for the prediction in durum and common wheat (De Girolamo et al., 2009). Even though a higher accuracy for their models might be needed for implementation at the guideline value, the wide sample variety employed for model development underlines the potential of their proposed IR-based mycotoxin prediction approach. Gaspardo et al. showcased for the first time that NIRS can be used to predict FUMs in ground corn samples. They reported an RMSEP of 0.917 mg kg^{-1} using a test set (n = 25) for external validation of their model, thus achieving satisfactory predictive performance (Gaspardo et al., 2012). Della Riccia et al. underlined the capability of NIRS to predict FUM at regulatory limits, concluding that larger sample sets may be beneficial (Della Riccia Giacomo, 2013). De Girolamo et al. also developed a model for the prediction of DON in durum wheat based on NIRS using a large sample set (n = 500). They report that they could not obtain a satisfying quantitative model, as the RMSEP of 1977 μg kg^{-1} was within the same order of magnitude as the EU guideline value for unprocessed durum wheat of 1750 μg kg^{-1}. However, by using linear discriminative analysis, they could classify 90% of the validation samples correctly at a threshold of 1400 μg kg^{-1} (De Girolamo et al., 2014). Kaya-Celiker et al. (2014) demonstrated that artificially inoculated peanuts could be discriminated according to their AFLA content at a threshold of 20 and 300 μg kg^{-1} AFLA, respectively. Levasseur-Garcia et al. (2015) reported a model that could classify corn samples (n = 117) from Denmark, France, Hungary, the Netherlands, and Poland at the EU regulatory limit for FUM of 4000 μg kg^{-1} with an accuracy of 82%. In addition, Levasseur-Garcia & Kleiber (2015) demonstrated in another manuscript that whole kernel corn samples from six different countries and years could be used to establish discriminative models for DON and FUM determination at the regulatory limits. De Girolamo and her co-workers also reported two models for the rapid screening of OTA in wheat (n = 255) using NIRS and MIRS. Their method has an impressive false compliant rate during classification of 6% (De Girolamo, von Holst, et al., 2019). Kos et al. (2016) reported the possibility to discriminate corn samples at a threshold of 500 μg kg^{-1} for DON in corn (n = 110) with 85% accuracy and peanuts (n = 92) contaminated with AFLA B_1 at a threshold of 8 μg kg^{-1} with an accuracy of 77% using ATR MIRS. A model with an accuracy of 92% for the discrimination of corn samples (n = 183) contaminated with DON at the threshold of 1250 μg kg^{-1} based on an ANN, among other methods was reported by Öner et al. (2019). This manuscript underlines the capabilities of classic ATR MIRS for mycotoxin screening and indicates the readiness of this method for real-life applications. However, the small sample size of a few mg necessary for ATR spectroscopy requires finely ground homogenous samples. Models for the prediction of DON in barley samples (n = 60) from 3 different regions in Brazil were reported by Caramês et al. (2020). They showcased that when selecting certain wavelengths, the robustness and accuracy of the reported PLS models significantly improved, resulting in excellent predictive performance. S. Zhang et al. (2021) showed that by combining machine vision with NIRS, 93.55% of wheat samples (n = 190) could be classified correctly. Tyska et al. (2021) explored NIRS to quantitatively predict FUMs and ZEN in Brazilian corn samples (n = 676). They found no significant statistical difference between the values obtained with the LC-MS/MS reference method and the ZEN values predicted by NIRS for their test set.

In the literature, it has been suggested that IR spectroscopy as rapid mycotoxin screening methods is limited to classify samples into highly or low contaminated samples at a chosen limit (Levasseur-Garcia, 2012). The publications listed in Table 6 underline this statement at first glance, as for some of the reported quantitative models, the RMSEP is relatively high. Gaspardo et al. (2012) obtained an RMSEP of 0.917 mg kg^{-1} for prediction of FUMs in corn. Tyska et al. (2021) reported an RMSEP of 69.7 μg kg^{-1} for ZEN in corn and 659 μg kg^{-1} for FUMs in corn. Caramês et al. (2020), on the other hand, reports an RMSEP of 160.76 μg kg^{-1} for prediction of DON in barley. In general, comparing the RMSEP of different publications is complex since the RMSEP is influenced by the concentration range used during evaluating the predictive performance of the reported model. The RMSEP obtained by a model aiming at the prediction of a mycotoxin with a higher legal limit, like FUMs in unprocessed corn (4000 μg kg^{-1}), will usually be higher than the RMSEP for models predicting the DON (1750 μg kg^{-1}) or ZEN (350 μg kg^{-1}) contamination in the same matrix. Therefore, the RMSEP must be evaluated with respect to the overall working range of the prediction model, which is defined by the legal limit of the mycotoxin. In addition to the RMSEP of 69.7 μg kg^{-1} for ZEN in corn, Tyska et al. (2021) reported that they did not find a significant difference between the prediction of mycotoxin values with NIRS and the corresponding LC-MS/MS

measurements. This challenges the statement that IR spectroscopy can only be used for classification at a defined threshold and indicates that quantitative screening is feasible, even though the RMSEP seems high compared to the legal limit. In the end, for further clarification, validation studies according to regulatory requirements should be performed to underline the applicability of IR methods for either classification or quantitative mycotoxin prediction.

6 | CONCLUSION, TRENDS, AND OUTLOOK

IR spectroscopy has demonstrated its applicability for the screening of certain mycotoxins in agricultural samples. Rapid detection for contamination with *Fusarium* toxins appears to be the most promising example for the implementation of an alternative IR spectroscopic approach within mycotoxin analysis.

Models for DON in corn and wheat exist, but prediction of DON seems to be more challenging than the determination of FUM or ZEN in corn. Levasseur-Garcia (2012) concluded the opposite. She stated that the prediction of DON contamination might be less challenging than the prediction of FUMs. Levasseur-Garcia et al. also reported a weak correlation between FUMs content and fungal biomass found in corn (Levasseur-Garcia, 2012, 2015). Several publications using IR spectroscopic methods to analyze DON levels in crops found similar trends, as it was reported that DON does not correlate well with other fungal metabolites such as ergosterol or fungal infestation symptoms (Dowell et al., 1999; Femenias, Bainotti, et al., 2021; Jin et al., 2014). This mismatch between fungal biomass and mycotoxin contamination could be also attributed to the challenges faced during the analysis of ergosterol. Caramês et al. (2020) reported that DON occurrence in Brazilian barley differs due to geographical origin of the sample. In the end, the better prediction of FUMs, DON, or ZEN might also be linked with the chosen sample pool and the used reference method. To conclude, further research is needed to provide a better understanding of the relation between *Fusarium* toxin production, fungal biomass, and sample changes in the context of developing IR-based screening methods. Prediction of AFLA contamination was also subject to IR spectroscopic studies. Models aiming to discriminate samples at AFLA guideline values look promising, but until now have lacked the accuracy required for implementation. This is due to the low regulatory limits and the complex matrix of the investigated commodities (i.e., ground peanut) (Kaya-Celiker et al., 2014; Kos et al., 2016). Discrimination of samples according to their contamination with OTA on the other hand seems to be feasible, as high classification accuracies have been reported using naturally contaminated samples (De Girolamo, von Holst, et al., 2019).

Based on the performance achieved for the rapid (routine) analysis of mycotoxins at regulatory limits, IR spectroscopic methods can be divided into two categories (Table 6).

- Category A: Those NIRS and MIRS methods fall into this category, for which the readiness for the routine analysis of mycotoxins has already been demonstrated. Publications in this category of methods rely on a wide variety of naturally contaminated samples and report quantitative mycotoxin prediction or aim at the classification of samples at a legal limit. Accurate quantitative prediction of ZEN and FUMs in corn and DON in barley using NIRS has been demonstrated (Caramês et al., 2020; Tyska et al., 2021). Methods aiming at the classification of high and low contaminated samples with an accuracy bigger than 90% include the prediction of DON in wheat and corn and OTA in wheat using NIRS and MIRS (De Girolamo, von Holst, et al., 2019; Öner et al., 2019; B. Zhang et al., 2021).
- Category B: Those methods fall into this category, which show good promise for routine analytical applications in the near future. Such methods rely either on NIRS, MIRS, or HSI. Some of these methods are based on studies which either rely on a small sample pool or on inoculated samples for model development. A larger set of naturally contaminated samples needs to be included to prove the robustness of the reported models. This category of methods also includes studies using relatively big, naturally contaminated sample sets, but the reported models did not achieve the satisfying predictive performance required for implementation in a routine analytical setting. Further work is required to increase the accuracy of the reported models by increasing the number of samples or by exploring other algorithms for model development. Prediction of DON in corn and wheat, FUMs in corn, or AFLA in peanuts fall all into this category (De Girolamo et al., 2014, 2009; Della Riccia Giacomo, 2013; Femenias, Gatius, et al., 2021; Gaspardo et al., 2012; Kaya-Celiker et al., 2014; Kos et al., 2004, 2003, 2007, 2016; Levasseur-Garcia et al., 2015; Levasseur-Garcia & Kleiber, 2015).

Category A publications demonstrate the applicability of IR spectroscopy, but none of the proposed methods have been fully in-house validated yet, which should also include appropriate means of external quality assurance, such as the analysis of certified reference materials or by participation in an interlaboratory comparability study. The indirect nature of IR spectroscopic methods for rapid mycotoxin determination renders them all screening

methods as defined by EU regulations (Commission, 2002). Such mycotoxin screening methods aim at the classification of the analyzed samples into either "suspect" or "negative" samples at a screening target concentration with a certainty of 95% (Commission, 2014). Von Holst and Stroka summarized those requirements and provided examples on the validation of screening methods either using a numerical or qualitative output of an analytical method according to European Commission (EC) criteria (Von Holst & Stroka, 2014). Lattanzio et al. (2016) published a case study on the validation of an LFA for DON in wheat according to these EC requirements. They showed that the employed LFA kit can be used to screen for DON in wheat at EC regulatory limits. A strong correlation ($R = 0.87$) was found by Lattanzio et al. between the LFA and the LC-MS/MS measurements for DON in wheat. The very strong correlation ($R^2 = 0.98$) reported by Tyska et al. (2021) between the prediction of ZEN in corn using NIRS and the corresponding LC-MS/MS measurements on the other hand indicates that IR spectroscopic methods might also fulfill such EC validation requirements during a pilot study.

IR spectroscopy might not only be used for screening of mycotoxins in bulk samples, but also to reduce mycotoxin concentration in agricultural commodities and thereby directly improving food and feed safety. The nondestructive character of IR spectroscopy allows for the analysis of every individual kernel intended for human or animal consumption. The potential to identify single highly contaminated kernels or nuts by SKNIR or HSI has been demonstrated by a multitude of publications (Tables 4 and 5). By leveraging the potential of SKNIR for grain-sorting applications, the bulk concentration of AFLA, FUM, and DON can be reduced (Delwiche et al., 2005; Pearson et al., 2004; Saito et al., 2009; Stasiewicz et al., 2017). Salvaging noncontaminated kernels from batches declared unusable as food or feed to counteract crop yield reduction due to fungi infestation might be a further promising area for implementation of IR spectroscopy. Realization of such a massive undertaking like sorting tons of grain kernels is aided by the ongoing miniaturization of NIR spectrometers toward coin-sized devices and might help in the food crisis to come.

On-site or in-field mycotoxin determination is the common goal for all rapid screening methods. For many years, there has been an ongoing trend in spectroscopy toward miniaturization (Crocombe, 2018). Combined with the ease of sample handling as well as the straightforward character of spectrometer operation, IR spectroscopy seems to be the ideal candidate for in-field or on-site analysis. A multitude of portable IR spectrometers exist and the analysis of mycotoxin contamination using a handheld NIR device has been showcased recently (Shen et al., 2022). However, implementation at regulatory limits is still to be achieved. Several handheld spectrometers can now be operated using only a smartphone app or with software installed on a tablet or laptop. Thereby, cloud-based data evaluation schemes are enabled, facilitating on-site analysis. This highlights another technological advance toward miniaturization and in-field implementation. However, to run these applications or software, a stable internet or WIFI connection is required, thus adding another layer of complication for remote in-field locations. Therefore, not only the spectrometer and data analysis, but the whole analytical procedure must be robust. Currently, the most promising applications require the sample to go through a mandatory grinding step (Table 6). Thus, methods with no or limited sample preparation must be further refined, to allow for true on-site implementation and further research in this area should be conducted. Portable spectrometers may also be useful for lab-based analysis, as their space requirements are minimal. The modular characteristics of these devices suggest their integration with other laboratory equipment like mills and allows for in-line analysis during sample handling schemes in routine analytical laboratories. In general, IR spectroscopy holds great potential to aid other routine analytical methods as a preceding screening tool, to lift the burden from other more complex methods such as LC-MS/MS.

In MIRS, the commercial availability of high-power laser-based radiation sources like QCLs or supercontinuum lasers led to a multitude of novel implementations, which challenge the dominance of classic FT-IR spectrometers relying on thermal emitters (Schwaighofer et al., 2017; Zorin et al., 2022). QCLs and interband cascade lasers facilitate the downsizing of MIR spectrometers and thus hold great potential for portable devices. Currently, the use of QCLs is focused on gas phase and liquid phase analysis either targeting process analytical problems or life science applications (Schwaighofer et al., 2017). Few applications of QCLs for the analysis of agricultural products have yet been shown. Among them the analysis of bovine milk by a QCL-based spectrometer indicates the potential of novel MIR spectroscopic concepts for the analysis of agricultural commodities (Kuligowski et al., 2017). QCL-based spectrometers have already been used for mycotoxin determination by their combination with waveguides (Sieger et al., 2017). It can be expected that the higher power of QCLs, compared to thermal emitters, will lead to the development of innovative MIR spectroscopic approaches. These approaches could possibly facilitate increased sensitivity and simplified application of the MIR spectral region for the analysis of mycotoxins in agricultural crops and thus, challenge the recent dominance of NIRS. The recently funded European Union project Photonfood pursues such approaches involving novel MIR sources to

develop a portable solution, which enables the on-site screening of mycotoxins. In the end, such developments enhancing MIRS might in the future facilitate the analysis of mycotoxins, including AFLAs, at the required regulatory limits and enable the screening of mycotoxins in more complex food and feed matrices.

Current IR spectroscopic methods summarized in this study rely on the indirect prediction of mycotoxin contamination using chemometric models. New data science tools such as deep learning algorithms (i.e., convolutional neural networks) have improved data analytics in recent years. These techniques have grown in popularity and can also aid spectra analysis. These new data analysis tools may be advantageous for the determination of mycotoxins with lower regulatory limits, as recent studies have already shown that deep neural networks can be applied to vibrational spectroscopy (Yang et al., 2019). Additionally, access to large cloud-based prediction models might help improving the performance of handheld spectrometers used for in-field measurements. Thus, these advances may provide an alternative to conventional chemometrics leading to improved model predictions, further facilitating the transition to practical analytical applications. Additionally, it must also be emphasized that, IR spectroscopic methods can not only be used for rapid mycotoxin analysis of agricultural bulk samples or to sort kernels, but also to study the nature of the fungal infection. Several authors reported a clear link between fungi-infected samples and shifts in the corresponding IR spectra (Dachoupakan Sirisomboon et al., 2013; Fernández-Ibañez et al., 2009; Zheng et al., 2020). However, band assignment using NIRS in complex samples like fungi-infected grain is difficult. By using MIRS, additional and easier to interpret information is obtained. Thus, MIRS can not only be used to predict mycotoxin contamination but also to study the alteration of the sample by mycotoxigenic fungi and therefore, help to understand the mechanism behind fungal infestation.

To conclude, the implementation of IR spectroscopy in mycotoxin routine analysis remains a challenge but appears to be feasible for a range of *Fusarium* toxins. Comprehensive validation and benchmarking of IR spectroscopic approaches against other rapid methods is the next logical step. The development of robust prediction models requires IR spectroscopic knowledge, a fitting sample pool, advanced chemometrics, and reliable reference analysis; thus, the broad knowledge base of several experts is essential. Once the obstacles of model development are overcome, the eco-friendly, nondestructive, and straightforward character of IR spectroscopy outshines other screening techniques. Moreover, IR spectroscopy can and should be applied for grain-sorting applications in the future to reduce the bulk mycotoxin concentration and to study the nature of fungal infections.

AUTHOR CONTRIBUTIONS

Stephan Freitag: Conceptualization; Investigation; Writing – original draft; Writing – review & editing. **Michael Sulyok**: Supervision; Writing – review & editing. **Natasha Logan**: Writing – review & editing. **Christopher T. Elliott**: Resources, Writing review & editing. **Rudolf Krska**: Resources; Supervision; Writing – review & editing

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CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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