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Fluorescence Imaging as a Non-Destructive Method for Aflatoxin Detection in Corn Kernels: Recent Advances and Challenges

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ABSTRACT

Fluorescence imaging has developed as a promising non-invasive method for identifying aflatoxin contamination in agricultural commodities, especially corn kernels. This paper examines current improvements in fluorescence imaging technologies, highlighting its potential to improve food safety through swift and precise detection of mycotoxins. The paper examines the basics of fluorescence, the necessary setup for optimal imaging, and the issues related to background fluorescence interference, sensitivity, and the construction of calibration models. Although there are some limitations, fluorescence imaging presents considerable advantages, such as cost-efficiency and the capacity to obtain concurrent spectral and spatial data. Proposed future research objectives include the validation of imaging systems using naturally contaminated samples, the optimization of imaging parameters, and the integration of machine learning techniques to enhance data processing. By overcoming existing constraints and utilizing technical progress, fluorescence imaging can serve as an essential instrument in the detection of aflatoxin contamination, hence enhancing food safety.

1. INTRODUCTION

Corn (*Zea mays*) is a staple crop globally, serving as a primary source of food, animal feed, and industrial products. Its productivity is significantly affected by various organisms, particularly fungi such as *Aspergillus flavus*, which is producing aflatoxins-highly toxic and carcinogenic compounds that pose serious health risks to humans and livestock (Benkerroum, 2020; Kerry *et al*., 2017; Yan & Wu, 2010; Yu *et al*., 2022). The prevalence of *Aspergillus flavus* is influenced by environmental conditions, including temperature and humidity, which can create favorable conditions for its growth and subsequent aflatoxin production (Budianto *et al*., 2022; Fountain *et al*., 2014; Medina *et al*., 2014). In tropical regions like Indonesia, characterized by high humidity, the risk of aflatoxin contamination in corn kernels is particularly pronounced (Budianto *et al*., 2022; Jallow *et al*., 2021; Udomkun *et al*., 2017).

Aflatoxins, especially aflatoxin B1, are regulated by food safety authorities due to their potential health hazards. The U.S. Food and Drug Administration has established a limit of 20 parts per billion (ppb) for total aflatoxins in food and feed products (Kerry *et al*., 2017). Similarly, the Food and Agriculture Organization (FAO) has set guidelines to mitigate aflatoxin risks in agricultural commodities, emphasizing the need for stringent monitoring and control measures (Udomkun *et al*., 2017). These regulations are crucial, as aflatoxins can cause acute and chronic health issues, including liver cancer, making their management essential for food safety (Kerry *et al*., 2017; Yu *et al*., 2022).

The spread of aflatoxins is exacerbated by climatic conditions, particularly in Indonesia, where high temperatures and humidity create an ideal environment for *Aspergillus flavus* proliferation (Budianto *et al*., 2022; Temba *et al*., 2021). The combination of these weather patterns can lead to increased aflatoxin levels in maize, particularly during the post-harvest phase when moisture level is often unregulated (Smith *et al*., 2016). Studies have shown that periods of drought followed by high humidity can significantly elevate aflatoxin contamination levels in crops (Hao *et al*., 2023; Umesha *et al*., 2016). This situation is particularly concerning for smallholder farmers in Indonesia, who lack the resources for effective grain drying and storage, further increasing the risk of contamination (Smith *et al*., 2016).

To detect aflatoxins, various analytical methods have been developed, including High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS), and Enzyme-Linked Immunosorbent Assay (ELISA) (Yao *et al*., 2022). While these methods are effective, they often require complex sample preparation and can be time-consuming, which may limit their applicability in rapid screening scenarios (Mitchell *et al*., 2016; Udomkun *et al*., 2017). Additionally, they typically involve destructive sampling, which can be a disadvantage in certain contexts where preserving the sample is essential (Chu *et al*., 2018a; Udomkun *et al*., 2017; Wang *et al*., 2010).

Emerging technologies, particularly advanced visual methods like fluorescence imaging, are revolutionizing aflatoxin detection. Fluorescence imaging, in particular, has gained significant attention due to its ability to non-invasively detect aflatoxin-contaminated crops with remarkable sensitivity and speed. This technique exploits the unique fluorescence properties of aflatoxins, which emit specific wavelengths of light when exposed to certain excitation wavelengths. By utilizing sophisticated fluorescence-transmission imaging systems, this technology significantly enhances the spectroscopic sensitivity, allowing for rapid and precise detection of aflatoxins even in highly contaminated kernels (Chavez *et al*., 2020; Ehrlich, 2014; Endre *et al*., 2023). Fluorescence imaging systems stand out by offering numerous advantages over traditional detection methods. They are non-destructive, allowing for real-time monitoring without the need to sacrifice or alter the sample. This is especially advantageous for large-scale screenings, where preserving the integrity of the agricultural products is critical. Additionally, fluorescence imaging provides faster results compared to conventional laboratory techniques, enabling on-site, real-time detection that can help farmers, traders, and food safety regulators make informed decisions to mitigate aflatoxin contamination (Chavez *et al*., 2020; Divakara *et al*., 2015).

Given these considerations, this review critically examines recent advancements in fluorescence imaging as a non-destructive technique for aflatoxin detection in corn kernels. The discussion encompasses the fundamental principles of fluorescence imaging, its comparative advantages over conventional analytical methods, existing challenges that hinder its broader implementation, and the potential integration of deep learning to enhance detection accuracy and efficiency. By synthesizing current research findings, this review aims to provide a comprehensive analysis of the feasibility, limitations, and future directions of fluorescence imaging in ensuring food safety and mitigating mycotoxin contamination in agricultural commodities.

2. METHODS

This research employs a systematic review methodology to analyze the advancements in fluorescence imaging technologies for detecting aflatoxins in agricultural products. The methodology consists of several key stages, including literature search, study selection, data collection, and analysis of research findings, which are detailed as the followsing.

2.1. Literature Search Strategy

2.1.1. Databases and Search Keywords

The literature search was conducted across multiple academic databases, including Scopus, PubMed, Web of Science, and Google Scholar. A combination of keywords was utilized to maximize the relevance of the search results, including "fluorescence imaging," "aflatoxins," "non-destructive detection," and "agricultural products".

2.1.2. Search Limitations

Publications were restricted to those published within the last 15 years to ensure the inclusion of the most recent technological advancements. Only articles published in English were considered. Studies focusing specifically on non-destructive methods and applications of fluorescence imaging for aflatoxin detection were prioritized.

2.2. Inclusion and Exclusion Criteria

2.2.1. Inclusion Criteria

This study includes research that investigates the application of fluorescence imaging for the detection of aflatoxins. Additionally, articles presenting quantitative experimental results related to aflatoxin detection are considered within the scope of this review.

2.2.2. Exclusion Criteria

Studies that are purely theoretical and lack direct experimental data, as well as those with incomplete or non-extractable data, are excluded from this review.

2.3. Data Extraction

2.3.1. Parameter Collected

Parameter that collected were optimal wavelength for aflatoxin detection, imaging techniques employed (fluorescence imaging), classification accuracy of the detection methods, and types of agricultural products tested for aflatoxin contamination.

2.3.2. Data Organization

Data were systematically collected and organized using a standardized table format, which included the following columns: - Author(s) - Year of Publication - Analysis Method - Experimental Results.

2.4. Analysis of Research Findings

The collected data were analyzed to identify trends in the effectiveness of fluorescence imaging technologies for aflatoxin detection. This included evaluating the sensitivity, specificity, and overall performance of different imaging techniques. Furthermore, the findings were synthesized to provide a comprehensive overview of the current state of fluorescence imaging technologies in the context of aflatoxin detection, highlighting both the advancements and the challenges faced in this field.

3. RESULTS AND DISCUSSION

In recent years, numerous studies have explored non-destructive image processing methods for detecting aflatoxin, an analytical tool developed since 2001 for early detection across various agricultural products. Digital image processing allows for the observation and analysis of objects without causing any damage. This technique involves capturing digital images that form a matrix representing light intensity at specific points, enabling the extraction of quality-related information from agricultural products. However, conventional image processing methods struggle to effectively detect aflatoxins due to their unique chemical properties, which are not efficiently captured under standard lighting conditions.

To address these limitations, fluorescence imaging technologies have been advanced to specifically target aflatoxin detection (Figure 1). Fluorescence imaging is particularly advantageous in identifying the unique fluorescence signature of aflatoxins, allowing for more accurate, non-destructive, and efficient detection compared to traditional imaging methods. This approach has proven effective in overcoming the challenges posed by conventional techniques, providing reliable results in real-time monitoring of agricultural products.

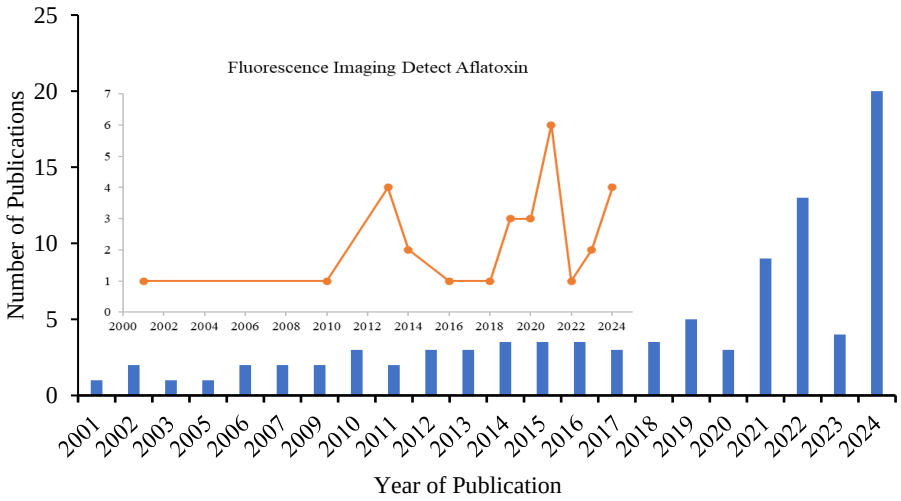

Figure 1. Annual publications on image processing techniques in agricultural products (blue bars), with a specific focus on fluorescence imaging for aflatoxin detection (orange line, inset graph) (based on articles indexed in Scopus)

The figure presented the number of annual publications related to image processing techniques in agricultural products, with a specific focus on fluorescence imaging for aflatoxin detection. The blue bars show the general trend in image processing publications, which have steadily increased over time. The inset graph (orange line) zooms in on the subset of publications specifically related to fluorescence imaging for aflatoxin detection. The data highlights a sharp rise in research output, particularly in recent years, signaling the growing importance of fluorescence imaging in detecting aflatoxins in agricultural commodities. This trend reflects an increasing recognition of the value of fluorescence imaging as a key tool for ensuring food safety and minimizing health risks associated with aflatoxin contamination.

3.1. Characteristics of Secondary Metabolites

There are six major aflatoxins: B1, B2, G1, G2, M1, and M2. These are highly oxygenated, naturally occurring heterocyclic compounds share a benzene ring structure, with variations in double bonds, ketonic groups, and hydroxylation positions that influence their solubility, epoxidation, and toxicity. Aflatoxin B1 (AFB1), produced by *Aspergillus flavus* and *Aspergillus parasiticus* under favorable conditions, is the most potent and toxic due to its double bond at carbons 8 and 9, a feature also present in G1 and M1 (Lien *et al*., 2019; Nazhand *et al*., 2020). AFB1 is an odorless, tasteless, and colorless compound that poses challenges for detection. It appears as pale yellow or white crystalline powder and exhibits blue fluorescence with a maximum emission at 425 nm and UV absorbance peaks at 223, 265, and 362 nm (Al-Jaal *et al*., 2019; Nazhand *et al*., 2020). Similarly, Aflatoxin B2 (AFB2), like AFB1, is produced by *Aspergillus* species. It is structurally similar to B1 but lacks the specific double bond at carbons 8 and 9. Although it is less toxic than B1, AFB2 also exhibits significant carcinogenic properties. AFB2 emits blue fluorescence under UV light, similar to AFB1, though with slight variations in intensity and peak emission (Balina *et al*., 2018; Yang, 2020).

In contrast, Aflatoxin G1 (AFG1) is a derivative of AFB1 and shares many structural similarities, with the key difference being a shift in the position of the double bond, resulting in a greenish-yellow fluorescence under UV light. AFG1 is also highly toxic and carcinogenic, and it poses significant risks to both humans and animals. It typically exhibits maximum excitation wavelengths between 360–370 nm (Balina *et al*., 2018; Yang, 2020). Unlike AFB1, Aflatoxin G2 (AFG2) is a derivative of AFB2. It also fluoresces greenish-yellow under UV light, though it is less potent and less commonly encountered in nature. The fluorescence properties and toxicological concerns related to AFG2 are comparable to those of AFG1, but its occurrence is generally less frequent (Balina *et al*., 2018; Yang, 2020).

In the case of Aflatoxin M1 (AFM1), this is a hydroxylated metabolite of AFB1 and is commonly found in the milk of animals that have consumed contaminated feed. AFM1 is considered a carcinogen, and while it is less potent than AFB1, it still poses significant health risks to consumers of dairy products. AFM1 also exhibits blue fluorescence under UV light, with similar emission characteristics to AFB1 but with subtle differences in intensity and peak wavelengths (Barikbin *et al*., 2015; Daşbaşı, 2022). Similarly, Aflatoxin M2 (AFM2) is a hydroxylated derivative of AFB2 and, like AFM1, can be found in the milk of animals exposed to contaminated feed. While it is less toxic than AFM1, it remains a significant concern in terms of food safety. AFM2 also fluoresces blue under UV light, although it tends to exhibit slightly different fluorescence intensity and wavelength characteristics compared to its parent compound, AFB2.

All six aflatoxins exhibit unique fluorescence properties under UV light, which aids in their identification and classification. B1 and B2 emit blue fluorescence, while G1 and G2 emit greenish-yellow fluorescence. These variations in fluorescence intensities allow for the differentiation of aflatoxins, though overlap can occur with natural compounds in agricultural products. Additionally, as aflatoxin content increases, the fluorescence intensity decreases, and peaks shift between 437 and 537 nm (Teena *et al*., 2013). These fluorescence characteristics, along with other physico-chemical properties, are essential for distinguishing different types of aflatoxins in contaminated products, as shown in Table 1.

Table 1. Physical and chemical properties of aflatoxins

Type	Physico-chemical Properties		Ref.
AFB1	IUPAC Name	(2,3,6aR,9aS)-2,3,6a,9a-Tetrahydro-4-methoxycyclopenta[c]furo[2,3-h]chromen-1,11-dione	IARC (2012);
	Molecular weight	312.277 g/mol	
	Melting point	268-269°C	
	Physical condition	Colorless pale-yellow crystals to solid or white powder; odorless	
	Solubility rate	16,14 mg / l pada 25°C; decreases at low temperatures; generally soluble in water and polar solvents	
	Stability	Stable to melting point; decomposed by UV irradiation in water/chloroform	
	Fluorescence emission	Concentrated fluorescent blue (λmax = 425 nm)	
	UV Absorbance	Absorbs at 223, 265 and 362 nm wavelengths	
	Mass spectrum	Identification by LC-MS; ESI ionization; precursor-type [M+H]+; m/z 313.071	
AFB2	IUPAC Name	(2R,3R,6aR,9aR)-2,3,6a,9a-Tetrahydro-4-methoxydifuro[3,2:2',3']chromen-1,11-dione	IARC (2012);
	Molecular weight	314.278	
	Melting point	269-270°C	
	Physical condition	Colorless pale-yellow crystals to solid or white powder; odorless	
	Solubility rate	Soluble in water and polar solvents	
	Stability	Stable to melting point	
	Fluorescence emission	Concentrated fluorescent blue (λmax = 425 nm)	
	UV Absorbance	Absorbs at 265 and 362 nm wavelengths	
	Mass spectrum	[M+H]+; m/z 314.073	
AFG1	IUPAC Name	(2,3,6aR,9aS)-2,3,6a,9a-Tetrahydro-6a,9a-dihydroxy-4-methoxyfuro[2,3-h]chromen-1,11-dione	IARC (2012); CAST (2013)
	Molecular weight	328.289	
	Melting point	267-268°C	
	Physical condition	Colorless pale-yellow crystals to solid or white powder; odorless	
	Solubility rate	Soluble in water and polar solvents	
	Stability	Stable to melting point	
	Fluorescence emission	Concentrated fluorescent blue (λmax = 450 nm)	
	UV Absorbance	Absorbs at 243 and 362 nm wavelengths	
	Mass spectrum	[M+H]+; m/z 328.076	
AFG2	IUPAC Name	(2R,3R,6aR,9aR)-2,3,6a,9a-Tetrahydro-6a,9a-dihydroxy-4-methoxydifuro[3,2:2',3']chromen-1,11-dione	IARC (2012); CAST (2013)

Type	Physico-chemical Properties		Ref.
	Molecular weight	329.290	
	Melting point	267-268°C	
	Physical condition	Colorless pale-yellow crystals to solid or white powder; odorless	
	Solubility rate	Soluble in water and polar solvents	
	Stability	Stable to melting point	
	Fluorescence emission	Concentrated fluorescent blue (λmax = 450 nm)	
	UV Absorbance	Absorbs at 265 and 362 nm wavelengths	
	Mass spectrum	[M+H]+; m/z 329.077	
AFM1	IUPAC Name	(2,3,6aR,9aS)-2,3,6a,9a-Tetrahydro-6a-hydroxy-4-methoxycyclopenta[c]furo[2,3-h]chromen-1,11-dione	Luis *et al*. (2016), Chen *et al*. (2018)
	Molecular weight	328.289	
	Melting point	254-256°C	
	Physical condition	Colorless to pale yellow crystalline powder	
	Solubility rate	Soluble in polar solvents	
	Stability	Stable in milk but decomposes upon UV exposure	
	Fluorescence emission	Concentrated fluorescent (λmax = 435 nm)	
	UV Absorbance	Absorbs at 223 and 333 nm wavelengths	
	Mass spectrum	[M+H]+; m/z 328.076	
AFM2	IUPAC Name	(2R,3R,6aR,9aR)-2,3,6a,9a-Tetrahydro-6a-hydroxy-4-methoxydifuro[3,2:2',3']chromen-1,11-dione	Luis *et al*. (2016); Finglas *et al*. (2008)
	Molecular weight	329.290	
	Melting point	254-255°C	
	Physical condition	Colorless to pale yellow crystalline powder	
	Solubility rate	Soluble in polar solvents	
	Stability	Stable in milk but decomposes upon UV exposure	
	Fluorescence emission	Concentrated fluorescent (λmax = 435 nm)	
	UV Absorbance	Absorbs at 223 and 333 nm wavelengths	
	Mass spectrum	[M+H]+; m/z 329.077	

3.2. Analytical Method

Several methods of analysis have been established and can be classified into three categories: chromatographic (Cernoch *et al*., 2012), immunochemical (Anfossi *et al*., 2011), and spectroscopic (Szulc *et al*., 2021). Each offers distinct advantages and limitations depending on factors such as sensitivity, cost, and sample preparation.

Chromatographic methods, including Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), and Liquid Chromatography-Mass Spectrometry (LC-MS/MS), provide high sensitivity and specificity. While TLC is cost-effective, it is less sensitive compared to HPLC, which offers better resolution but requires complex sample preparation and is more expensive. LC-MS/MS allows for highly sensitive detection of multiple mycotoxins but requires specialized equipment and is costly. On the other hand, Immunochemical methods, such as Enzyme-Linked Immunosorbent Assay (ELISA) and Radioimmunoassay (RIA), are faster and less expensive. These methods are suitable for high-throughput screening but suffer from lower sensitivity and potential cross-reactivity. Meanwhile, Spectroscopic methods, like fluorescence spectroscopy is rapid, non-destructive, and suitable for field testing with minimal sample preparation. However, their sensitivity can be impacted by background interference, limiting their use for detecting low levels of contamination.

The selection of an appropriate method is contingent upon the required sensitivity, speed, cost, and complexity of the sample matrix. Table 2 provide comparison of these methods based on detection limits, sample preparation requirements, and their ability to analyze multiple mycotoxins. Additionally, Table 3 summarizes the advantages and disadvantages of the methods discussed. Chromatographic techniques offer high precision but are costly and require extensive sample preparation. Immunochemical methods are faster and more affordable but exhibit lower sensitivity and are more prone to cross-reactivity. Spectroscopic methods are non-destructive and fast, making them ideal for field applications; however, their sensitivity is susceptible to background interference.

Table 2. Comparison of different aflatoxin analysis methods

Category	Methods	Sample price	Portable	AVM	LOD	Sample preparation	Ref.
Chromatographic	TLC	Medium	No	No	1-20 ng/kg	SPE	Marutoiu *et al*., 2004
Chromatographic	HPTLC	Medium	No	No	Pictogram	Extraction	Ramesh *et al*., 2013
Chromatographic	HPLC	Medium	No	No	0.008-0.014 µg/kg	IAC or SPE	Jaimez *et al*., 2000
Chromatographic	LC-MS/MS	Medium	No	Yes	0.8 µg/kg	Extraction	Cappiello *et al*., 1995
Chromatographic	RIA	Medium	No	No	1 µg/kg	Extraction	(Mahfuz *et al*., 2020)
Chromatographic	ELISA	Medium	No	No	0.006 µg/kg	Extraction	(Zheng *et al*., 2006)
Immunochemical	Immunodipstick	Medium	Yes	No	5 µg/kg	Extraction	(Mahfuz *et al*., 2020)
Immunochemical	Immunosensor	Medium	No	No	OWLS (0.5-10 ng/mL)		(Mahfuz *et al*., 2020)
Immunochemical	Electrochemical	Medium	No	No	1 fM	Extraction	(Ammida *et al*., 2004)
Immunochemical	VICAM	Expensive	No	No	-	-	(Mahfuz *et al*., 2020)

Note: TLC = Thin Layer Chromatography; HPTLC = High-Performance Thin-Layer Chromatography; HPLC = High-Performance Liquid Chromatography; LC-MS/MS = Liquid Chromatography-Mass Spectrometry; RIA = Radioimmunoassay; ELISA = Enzyme-Linked Immunosorbent Assay; VICAM = VIsual Immunoassay for Mycotoxins; LOD = Limit of detection; AVM = Analysis of various mycotoxins; SPE = Solid Phase Extraction; OWLS = Optical Waveguide Lightmode Spectroscopy.

Table 3. Advantages and disadvantages of various analytical methods in detecting aflatoxin

Methods	Pros	Disadvantages
Gas Chromato-graphy (GC)	Simultaneous analysis of mycotoxins, high sensitivity, can be automated, provides confirmation (MS detector).	Expensive equipment, expertise required, derivation required, problems in matrix interference, non-linear calibration curves, *response drifting*, *carry-over* effects from previous samples, variations in repeatability
TLC	A reliable counting method when combined with densitometry, more accurate and precise, comparable to the HPLC (HPTLC; OPLC) method, official reference technique for aflatoxin	Destructive to samples, largely replaced by HPLC for quantitative analysis of aflatoxins
HPLC	High sensitivity, high selectivity, high repeatability, short analysis time	Expensive equipment, specialized operators required, sample preparation by destructive methods
LC-MS	Simultaneous analysis of mycotoxins, capable of low limit detection (LC/MS/MS), provides confirmation, no derivation required.	Very expensive, special skills required, ionization-dependent sensitivity, matrix-assisted calibration curves (for quantitative analysis), lack of internal standards.
ELISA	Specific, fast and relatively easy to use, simple sample preparation, cheap equipment, low limit of detection, simultaneous analysis few samples, suitable for screening, semiquantitative or quantitative analysis possible, limited use of organic solvents	Possible cross-reactivity with related mycotoxins, possible false positives/negatives, matrix interference issues, narrow detection range, LC analysis to confirm required
Biosensor	Fast, no cleaning procedure, high selectivity, reproducibility, and sensitivity, ease of use, low cost and portability, self-contained, simple design.	The requirement for sample destruction.
Immunoaffinity assay	IAC in combination with liquid fluorometry is comparable to LC for aflatoxin determination, the official method.	Sample destruction; limited to analysis of total aflatoxin.

Source: (Mahfuz *et al*., 2020)

3.3. Digital Image

Digital imaging plays an essential role in the non-destructive inspection and monitoring of agricultural products, allowing for real-time analysis without damaging the products. The technology captures images based on the light that is reflected, emitted, or transmitted by the agricultural product. Light interacts with the product at different wavelengths, which is key to understanding how digital imaging applies to agricultural inspection.

Agricultural products, such as fruits, grains, and vegetables, interact with light in a way that can reveal important information about their quality, safety, and potential contaminants. The electromagnetic spectrum, as shown in Figure 2, includes a wide range of wavelengths, with visible light and infrared (IR) being particularly important in agricultural inspections. Visible light (VIS), ranging from 400 to 700 nm, is the range in which human eyes operate, but digital imaging systems can extend into other wavelengths, including UV light, infrared, and visible light are used to capture detailed images of the products (Adão *et al*., 2017; Oliveira *et al*., 2022).

Each wavelength of light interacts with agricultural products in a distinct manner, providing unique information. For instance, colors in the visible spectrum correspond to specific wavelengths of light: blue light has a wavelength of approximately 475 nm, green light is around 520 nm, and red light is at 650 nm (Adão *et al*., 2017). Color images illustrate an integration of three primary wavelength bands: red, green, and blue (Neittaanmäki-Perttu *et al*., 2015).While the human eye is responsive to the blue, green, and red parts of the spectrum, with each color exhibiting a distinct range that is stimulated significantly based on the wavelength of the emitted light. The colors visible by the human eye represent a limited segment of the electromagnetic spectrum, ranging from 400 to 700 nm (Figure 2).

Visible light (400–700 nm) is commonly used to detect external features such as surface defects, color variations, and mold growth. UV light makes use of the fluorescence properties of certain contaminants like aflatoxins. When exposed to UV light, substances like aflatoxins emit fluorescence that can be captured by the camera, allowing for the identification of contamination (Fujita *et al*., 2013; Gao *et al*., 2018; Zhu *et al*., 2016). Infrared light, particularly in the the near-infrared (NIR) and short-wave infrared (SWIR) ranges, is used to examine internal characteristics like moisture content, ripeness, and internal damage that may not be visible with visible light.

The infrared range (IR) (from 700 nm to several μm, including NIR and SWIR regions) is crucial for detecting internal properties of agricultural products. For example, NIR can be used to assess the moisture content or detect early signs of spoilage in fruits and vegetables (Güneş *et al*., 2013; Moreau *et al*., 2011). SWIR, however, provides a more detailed analysis, useful for detecting mold growth or other hidden defects beneath the surface, offering a non-invasive means to assess internal damage that visible light alone cannot capture (Khalid *et al*., 2018; Zhu *et al*., 2016).

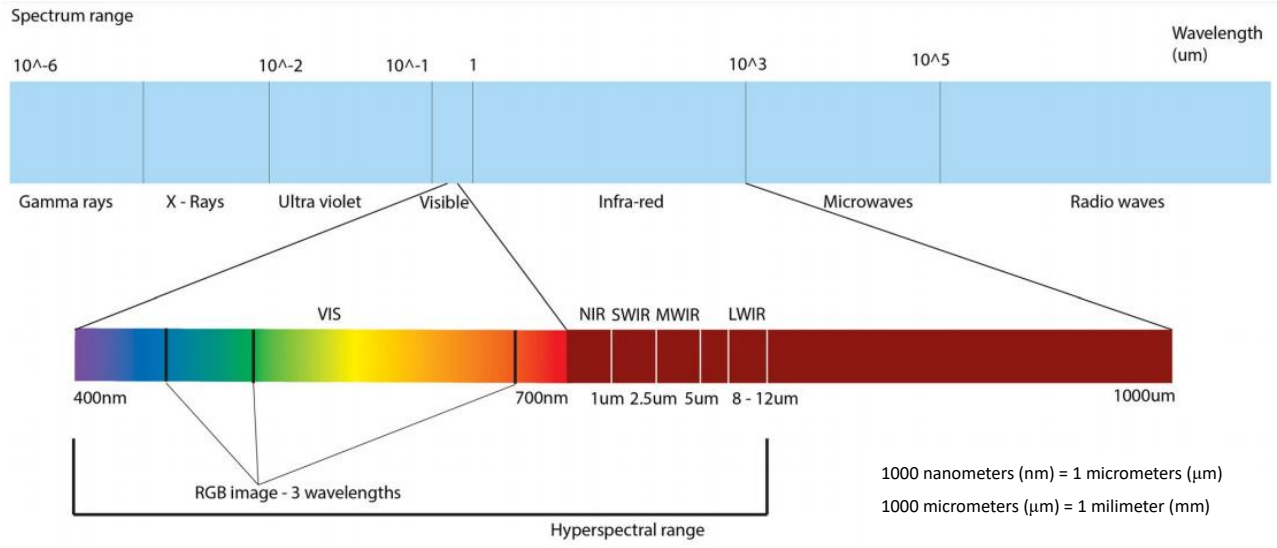

Figure 2. Electromagnetic spectrum featuring visible and infrared light (Lowe *et al*., 2013)

Once the images are captured using these varied wavelengths, the next crucial step is pre-processing. This step improves the quality of the captured image by removing noise and enhancing contrast, making key features more apparent for further analysis. After pre-processing, the image is segmented into different regions of interest (ROI), such as areas exhibiting contamination or surface damage. Important features, including color, fluorescence intensity, shape, and texture, are then extracted from these regions (Magnus *et al*., 2021; Sadimantara *et al*., 2024). These features provide data that can be analyzed using machine learning algorithms, which help classify the product based on quality. For instance, in fluorescence imaging, thy of fluorescence emission can be measured and correlated with contamination levels, such as the presence of aflatoxins (Fujita *et al*., 2013; Magnus *et al*., 2021). Machine learning models, trained on this data, can then automatically classify the agricultural products as either safe or contaminated.

This non-destructive method offers several benefitng the ability to inspect large batches of products quickly, without damaging them. Furthermore, digital imaging systems are capable of providing detailed insights into both the surface and internal quality of the products, improving the efficiency and reliability of quality control processes (Jallow *et al*., 2021; Kumar *et al*., 2017).

3.4. Fluorescence Imaging

Fluorescence imaging is a powerful optical technique that utilizes the phenomenon of fluorescence to visualize and quantify specific molecules within biological and agricultural samples. When a fluorescent compound absorbs light at a specific wavelength, it becomes excited and subsequently emits light at a longer wavelength. This property allows researchers to detect and analyze the presence of fluorescently labeled substances, making it a valuable tool in various fields, including biochemistry, molecular biology, and agricultural monitoring. The principle of fluorescence imaging is based on the excitation of fluorescent molecules by a light source, typically in the ultraviolet (UV) or visible spectrum. When these molecules absorb photons, their electrons are elevated to a higher energy state. The excited state is unstable, and the molecules quickly return to their ground state, releasing energy in the form of emitted light. This emitted light has a longer wavelength than the absorbed light due to energy loss during the excitation process. The fluorescence process typically occurs on a timescale of nanoseconds, allowing for rapid imaging and analysis (Göttfert *et al*., 2017). Aflatoxins exhibit specific fluorescence excitation peaks under UV light, aiding contamination detection in maize (Han *et al*., 2019). Higher contamination levels shift fluorescence peaks to longer wavelengths with reduced intensity (Zhu *et al*., 2016). High-speed dual-camera systems leveraging multispectral fluorescence imaging accurately identify contaminated maize samples. Effective aflatoxin detection depends on wavelengths strongly absorbed by aflatoxins, such as 200–250 nm and ~365 nm, resulting in high fluorescence intensity (Rasch *et al*., 2010; Smeesters *et al*., 2015) as shown in Figure 3.

The microspectrometer has been installed in a 90-degree configuration with the illumination system, in an optical setup that ensures a distance of 5 cm from the sample surface to the spectrometer as shown in Figure 4. The sample level in the petri dish is set to reach the edge so that the sample surface is at a fixed distance from the detector range (Bertani *et al*., 2020).

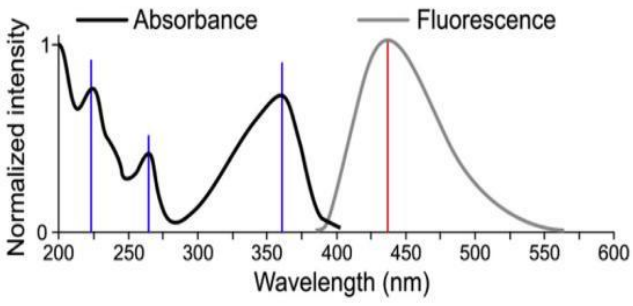

Figure 3. Absorbance and fluorescence spectra of aflatoxin B1 (Smeesters *et al*., 2015)

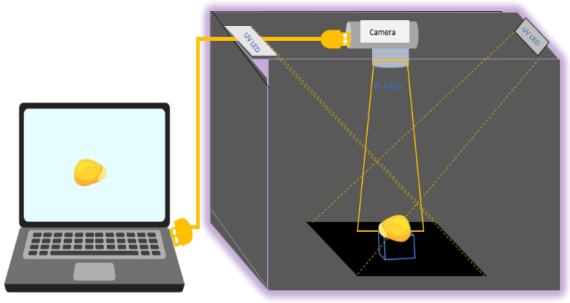

Figure 4. Schematic of fluorescence imaging setup. Adapted from Momin *et al*. (2023)

3.5. Classification of Aflatoxin Contaminated Food Products with Fluorescence Imaging

Aflatoxins, toxic secondary metabolites from *Aspergillus flavus* and *A. parasiticus*, are a persistent issue in maize. Rapid, non-destructive techniques like Fluorescence imaging offer potential for early detection and sorting of contaminated kernels as shown at Table 4. Fluorescence in the VNIR range (400–900 nm) has shown promising results for aflatoxin detection. Yao *et al.* (2010), observed a negative correlation between aflatoxin levels and fluorescence in blue-green bands ($r^2 = 0.72$). Subsequent studies confirmed longer fluorescence peak wavelengths in contaminated kernels and achieved classification accuracies up to 88% using binary code analysis (Yao *et al*., 2013b).

The application of spectral analysis in agricultural product inspection has become increasingly important, particularly for non-destructive methods aimed at detecting contaminants such as aflatoxins, assessing quality, and improving overall food safety. Several statistical methods have been utilized in conjunction with fluorescence imaging (FI) to interpret spectral data, classify agricultural products, and identify potential contaminants. Key techniques such as Multiple Linear Regression (MLR), Binary Encoding (BE), Principal Component Analysis (PCA), Factorial Discriminant Analysis (FDA), Partial Least Squares Discriminant Analysis (PLS-DA), Least Squares Support Vector Machines (LS-SVM), and Random Forest (RF) have shown promising results in enhancing the accuracy and efficiency of contamination detection, particularly when combined with fluorescence imaging for aflatoxin detection.

Table 4. Fluorescence imaging for detecting aflatoxin in food

Mode	Optimal wavelength range (nm)	Spectral range (nm)	Type of Aflatoxin	Aflatoxin Concentration (µg/kg)	Data Analysis	Model Performance Metrics	Ref.
Fluorescence	N/A	400-600	Not Specified (AF 13 strain)	0-14000	MLR	R^2_p= 0.72	(Yao *et al*., 2010)
Fluorescence	N/A	400-700	Not Specified	0-2000	DA	The Classification accuracy is 94.4%	(Yao *et al*., 2013a)
Fluorescence	437 and 537	400-900	Not Specified (AF 13 strain)	0-11000	BE	The classification accuracy is 87% for thresholds of 20 mg/kg and 88% for 100 mg/kg	(Yao *et al*., 2013b)
Reflectance	501 and 478	400-900	Not Specified (AF 13 and 36 strain)	0-8000	-	-	(Hruska *et al*., 2013)
Reflectance	N/A	1000-2500	AFB1	10-500	PCA FDA	The classification accuracy is >88%	(Wang *et al*., 2014)
Reflectance	N/A	1000-2500	AFB1	0-3800	PCA	The classification accuracy is 92%	(Wang *et al*., 2015)
Reflectance SWIR	N/A	1100-1700	AFB1	0-1000	PLS-DA	The classification accuracy is 97%	(Kandpal *et al*., 2014)
Fluorescence	501	399-701	Not specified	0-2662	LS-SVM	The classification accuracy is 90-95.3%	(Zhu *et al*., 2016)
Reflectance	701	461-877					
Reflectance and fluorescence	N/A	304-1086	Not Specified	0-20000	Random forest	The accuracy is approximately 95% with 86% sensitivity and 97% specificity	(Cheng *et al*., 2019)
Fluorescence	Ex: 365	400-2500	Not specified	10-1000	SVM	The classification accuracy is 89.1%	(Kim *et al*., 2023)
Fluorescence	Ex: 365 Em: 420-480	N/A	AFB1	0 - 320.2	DNN	The classification accuracy is between 84.7% and 93.0%	Bertani *et al*., 2023
Fluorescence	Ex: 365	N/A	AFG1	83.1	CNN	The classification accuracy is 96%	(Sadimantara *et al*., 2024)

Note: MLR: Multiple Linear Regression, PLS: Partial Least Squares, BE: Binary Encoding, PCA: Principal Component Analysis, R2p: Determination Coefficient for Prediction, FDA: Factorial Discriminant Analysis, LS-SVM: Least Squares Support Vector Machines, ANN: Convolutional Neural Network, DNN: Deep Neural Network

PCA is a widely used statistical technique for reducing the dimensionality of spectral data. By transforming the data into orthogonal principal components (PCs), PCA helps retain the most significant features while minimizing noise and irrelevant information. This technique is particularly useful when dealing with high-dimensional data, such as fluorescence spectra, which contain multiple variables and intricate patterns. Recent research by Hruska *et al.* (2013) demonstrated the effectiveness of PCA in identifying aflatoxin contamination in maize kernels based on spectral shifts observed in fluorescence imaging (Hruska *et al*., 2013). The study found that PCA significantly simplified the complex fluorescence spectra, making it easier to detect contamination. Similarly, Zhu *et al.* (2016), integrated fluorescence and reflectance data using PCA, achieving classification accuracies of 90–95% in maize contamination detection. They found that the germinal side of maize kernels exhibited superior performance, indicating that certain areas of the product provide more reliable spectral information for contamination detection (Zhu *et al*., 2016).

PLS-DA, a combination of Partial Least Squares Regression (PLSR) and Discriminant Analysis, is widely used for classification tasks, particularly when the data is highly multicollinear. PLS-DA maximizes the variance between different classes, which is critical for distinguishing subtle differences in contamination levels. Wang *et al.* (2015) successfully used PLS-DA to identify key wavelengths, such as 1729 nm and 2344 nm, for the characterization of Aflatoxin B1 (AFB1) contamination in maize kernels (Wang *et al*., 2015). Their study achieved classification accuracies between 88% and 96.9%, showing that PLS-DA could effectively detect AFB1 contamination from fluorescence spectral data. This highlights the robustness of PLS-DA in detecting contaminants and its ability to handle complex fluorescence imaging data efficiently.

LS-SVM is an enhanced version of traditional Support Vector Machines (SVM), using a least-squares cost function to solve the optimization problem. This method has been found to be particularly effective for non-linear classification, making it well-suited for the complex relationships present in fluorescence spectra. In a study by (Zhu *et al*., 2016), LS-SVM was used to classify aflatoxin-contaminated maize based on fluorescence imaging data from near-infrared (NIR) spectra. The study achieved an impressive 96% classification accuracy, demonstrating that LS-SVM is highly effective for classifying agricultural products based on spectral data. The method's ability to handle complex, non-linear relationships between spectral features and contamination levels makes LS-SVM a valuable tool for contamination detection in fluorescence imaging applications.

Random Forest (RF) is an ensemble learning method that creates multiple decision trees and combines their results to improve classification accuracy. This method is particularly useful when working with large, high-dimensional datasets, which are common in fluorescence imaging data. In Chu *et al*. (2018b), RF was used to classify wheat grain quality based on fluorescence imaging data, achieving 92% classification accuracy (Chu *et al*., 2018b). This study demonstrated the effectiveness of RF in handling the complexity of fluorescence spectra, where multiple features (such as color, fluorescence intensity, and texture) need to be processed simultaneously. RF is particularly advantageous because it is less prone to overfitting compared to individual decision trees and can effectively handle missing data, making it a robust choice for large-scale agricultural inspections.

Binary Encoding (BE) is an optimization technique often used for feature selection. In the context of fluorescence imaging, BE helps identify the most relevant spectral features by converting categorical data into binary form, improving the performance of classification models. Yao *et al*. (2013b), demonstrated the application of BE for selecting relevant spectral features in the detection of aflatoxins in grains (Yao *et al*., 2013b). By optimizing the feature set, BE improved the classification accuracy of fluorescence imaging models, helping to identify the most significant wavelengths related to contamination. This study illustrates the importance of feature selection in improving the accuracy and efficiency of fluorescence-based contamination detection.

Fluorescence imaging combined with statistical methods such as PCA, PLS-DA, FDA, LS-SVM, RF, and MLR provides a robust framework for aflatoxin detection in agricultural products. Each method offers distinct advantages, such as data reduction, classification accuracy, and the ability to handle multivariate and non-linear data. While PCA and PLS-DA excel in simplifying data and identifying key wavelengths for contamination detection, methods like LS-SVM and RF offer high classification performance, especially with large datasets. The integration of these statistical techniques with fluorescence imaging has shown considerable promise for improving the speed and accuracy of agricultural inspections, ensuring food safety, and enhancing the overall quality control processes. Continued research is essential to optimize these methods and enable their large-scale application in agricultural industries.

3.6. Limitations

Fluorescence imaging techniques demonstrate potential for classifying aflatoxin-contaminated food products and healthy grains, yet several challenges and limitations must be addressed. For instance, many studies rely on artificially inoculated samples with *Aspergillus flavus* conidia rather than naturally contaminated grains. This reliance raises the possibility that the fluorescence signals primarily reflect fungal presence rather than aflatoxin levels, especially since there is no direct linear relationship between fungal infection and the production of secondary metabolites like aflatoxins (Hruska *et al*., 2017). Consequently, results from these studies may not accurately represent contamination levels in real-world food products, where aflatoxin concentrations are typically within acceptable regulatory limits (2–20 µg/kg) (Wacoo *et al*., 2014; Yao *et al*., 2015).

Moreover, low fluorescence signal intensity can result in significant errors when recording maximum fluorescence intensity and emission peaks, particularly in samples with minimal contamination (Bartolić *et al*., 2022). In addition, Internal contamination of imaging instruments and cross-contamination during bulk sample analysis can also lead to false positives, further complicating the detection process (Bartolić *et al*., 2022). Furthermore, agricultural products often contain naturally fluorescent compounds whose emission spectra may overlap with those of aflatoxins, thereby obscuring the accurate identification and quantification of contamination.

However, higher contamination levels do not always equate to better detection sensitivity. For example, internal contamination within corn kernels, such as deeply embedded aflatoxin deposits, may affect fluorescence signal detection, since signal strength often depends on the contamination's location (Hruska *et al*., 2017; Yao *et al*., 2012). While it is assumed that the system's performance should remain unaffected by internal contamination, inconsistencies in signal response have been observed (Smeesters *et al*., 2016).

Additionally, the color of the sample may also influence fluorescence imaging results. Variations in the sample's surface color or pigmentation can alter the absorption and emission properties of fluorescence, potentially causing inconsistent or inaccurate results (Bartolić *et al*., 2022; Bertani *et al*., 2020). Thus, this issue underscores the importance of developing robust calibration techniques to account for such variability (Yao *et al*., 2023).

Another limitation is that fluorescence-based detection systems are sensitive to the operational conditions and design of the equipment. For instance, poorly maintained or contaminated systems can skew results, and cross-contamination is especially problematic when screening bulk samples (Chavez *et al*., 2020; Wacoo *et al*., 2014). For example, fluorescence imaging systems designed for bulk corn kernels may inadvertently detect fluorescence signals from external contaminants or environmental factors, thereby leading to false positives (Bartolić *et al*., 2022; Smeesters *et al*., 2016).

Despite these challenges, fluorescence imaging remains a promising tool for aflatoxin detection. To address these limitations, refinements in system design, such as advanced filtering to isolate aflatoxin-specific signals, improved calibration methods, and integration with complementary detection technologies, can enhance accuracy and reliability. Therefore, further studies are needed to optimize these systems for real-world applications, ensuring they can reliably detect contamination at regulatory limits and provide consistent results across diverse agricultural commodities.

3.7. Opportunities and Challenges

Overall, fluorescence imaging presents a promising non-invasive method for detecting mycotoxin contamination in agricultural commodities, offering a lower cost per sample compared to traditional analytical methods. This is due to the fluorescence imaging techniques can be implemented at a lower cost per sample than methods such as HPLC or mass spectrometry, making them accessible for widespread use in agricultural monitoring (Jacobson, 2024). Furthermore, fluorescence imaging allows for the simultaneous acquisition of spectral and spatial features, providing comprehensive data on the condition of agricultural products. As a result, this capability enhances the potential for early detection of aflatoxin contamination (Singh & Prasad, 2018). Fluorescence imaging has the potential to serve as an early detection tool for predicting the health of corn kernels and seeds, enabling timely interventions to mitigate contamination risks (Mateus *et al*., 2021).

A key advancement in fluorescence imaging is its integration with machine learning algorithms, particularly deep learning models, to enhance classification accuracy. Recent studies have demonstrated that combining fluorescence imaging with deep learning improves the discrimination of contaminated and uncontaminated samples by extracting subtle spectral features that may not be discernible using conventional analytical techniques (Li *et al*., 2020). Deep learning approaches, including convolutional neural networks (CNNs) and support vector machines (SVMs), have shown considerable success in classifying fluorescence spectral data with high accuracy. However, most of these studies have been conducted under controlled laboratory conditions, limiting their applicability to real-world agricultural environments. Future research should therefore focus on developing robust calibration models that account for environmental variability, including differences in lighting conditions, moisture content, and sample heterogeneity (Qin *et al*., 2020).

Despite its potential, fluorescence imaging faces several challenges that must be addressed to enhance its reliability and applicability. One major limitation is the reliance on artificially inoculated samples in experimental studies, which may not accurately reflect real contamination scenarios (Levasseur-Garcia, 2018). Future studies should compare corn samples contaminated with aflatoxin derived from *Aspergillus flavus* with pure aflatoxin, which is typically used as an analytical standard. Such a comparison is crucial for validating detection methods (Otto *et al*., 2020). Additionally, the presence of secondary metabolites in agricultural products may interfere with fluorescence signals, leading to false-positive or false-negative results. Therefore, optimizing the selection of excitation and emission wavelengths (particularly > 365 nm) can minimize spectral interference and improve detection specificity (Abdallah *et al*., 2018).

Another critical challenge is the development of universal calibration models to accommodate variations in biological activity, crop variety, and environmental conditions. Fluorescence signal intensity can be influenced by factors such as grain composition, pigmentation, and storage conditions, which may lead to inconsistencies in detection accuracy (Omar *et al*., 2020). Addressing these challenges requires the expansion of training datasets and the incorporation of advanced statistical techniques, such as discriminant analysis and regression models, to improve predictive performance and adaptability across different agricultural settings (Zhang *et al*., 2018).

Given these considerations, integrating fluorescence imaging with deep learning represents a promising direction for improving aflatoxin detection. Deep learning techniques can enhance fluorescence imaging by automating feature extraction, reducing background noise, and refining classification models for real-time application. Future research should explore hybrid approaches that combine fluorescence imaging with complementary detection technologies to further enhance sensitivity and specificity. By addressing these technical limitations and leveraging advancements in artificial intelligence, fluorescence imaging has the potential to become a highly reliable and scalable solution for ensuring food safety and mitigating mycotoxin contamination in agricultural commodities.

4. CONCLUSION

The effectiveness of fluorescence imaging for detecting aflatoxin contamination in corn relies on the careful selection of hardware tailored to the specific fluorescent characteristics of the kernels. Key factors include the use of a UV light source emitting wavelengths around 365 nm, optimal for exciting aflatoxin fluorescence, and a sensitive camera to capture emissions typically observed between 425 and 450 nm. Despite its potential, several challenges persist, such as background fluorescence interference and the need for robust calibration models to account for biological variability. Future advancements, including the integration of machine learning for data analysis and the development of portable imaging devices, promise to enhance detection capabilities. Continued research should focus on validating these systems with naturally contaminated corn samples and optimizing imaging parameters. By addressing these challenges, fluorescence imaging can become a vital tool for ensuring food safety and monitoring aflatoxin contamination in agricultural products.

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