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RESEARCH ARTICLE

Rapid Detection of Mycotoxins in Food Samples Using a Portable Bioanalytical Device

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Aflatoxins and ochratoxins are toxic compounds formed by fungi, which invade food crops and other agricultural products. A continuous check of mycotoxin levels in foods is crucial to reduce their associated hazards. This study focused on examining mycotoxin presence in corn, peanuts, figs and wheat as well as rice. Aflatoxin B1 was assayed by the AOAC method, ochratoxin A was determined by HPLC, deoxynivalenol was analyzed by VIS, and fumonisin B1 was tested by the method of AOAC. The average quantity of aflatoxin B1 found in the corn sample was 15.2 µg/kg with a low degree of variation reflected by the standard deviation of 0.5 µg/kg, and the coefficient of variation of 3.3%. It was also observed that peanuts had a higher aflatoxin B1 mean value of 22.3 µg/kg; thus, this commodity should be routinely screened. Ochratoxin A, at a level of 8.7 µg/kg, was detected in figs and can lead to progressive kidney disease upon consumption. Wheat flour samples also had deoxynivalenol (10.4 µg/kg) which is a fusarium mycotoxin and can pose some health risks if ingested. Fumonisin B1, from fusarium, was identified in rice and is toxic in large quantities; in rice, the concentration was 5.8µg/kg. Spike recovery analysis also confirmed the efficiency of mycotoxin detection and estimation. The mean percentage of extraction and recovery of AFB1 was 97.5%, OTA, 93.0%, and DON, 94.0%. Data from this study shows that mycotoxins were present in all the samples. Therefore, it is recommended that regular surveillance of agricultural products, particularly peanuts, should be conducted to prevent high population threats.

Keywords: mycotoxins: aflatoxin, ochratoxins, deoxynivalenol, fumonisin, food safety

Introduction

Statistics show that mycotoxins are toxic compounds produced by certain fungi that can easily infest agricultural produce and processed foods which are a potential danger to human health and food safety (Bennett & Klich, 2003). Some of the major mycotoxins of interest are aflatoxins, OTA, fumonisins, zearalenone, DON and patulin (Man et al., 2017). These mycotoxins may produce health impacts on humans and animals, including hepatotoxicity, nephrotoxicity, teratogenicity, immunosuppression, and high-dose acute toxicity (Xu et al., 2020). Aflatoxins have also been categorized as group 1 human carcinogens from the IARC and some have also been classified as other mycotoxins (Awuchi et al., 2021).

Aflatoxin is a threat to food safety, and it is costly since the products affected can be seized or recalled due to the restricted threshold (Abraham et al., 2012). Recent research points to mycotoxins causing food losses ranging between 5 and 10 percent of global production (Mahato et al., 2019). These are to prevent pre-harvest mycotoxin contamination and reduce post-harvest mycotoxin levels down to the barest minimum levels. On the other hand, surveillance programs have not lost their significance in identifying contaminated lots and ensuring that they are not supplied to consumers and or used for animal feed purposes (Dico et al., 2022).

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Mycotoxin analysis is a complex process and encompasses screening tests, which are easy to perform and may yield results within a short period and confirmatory analytical tests, which are accurate, sensitive and specific and may take a longer time to produce results (Patel et al., 1996). Simple diagnosis techniques include enzyme-linked immunosorbent assays (ELISA) and lateral flow devices (LFDs; Saha et al. 2021). These are rapid, inexpensive and often simple to perform on-site tests that yield qualitative or semi-quantitative results in minutes (Kon et al., 2022). However, with current technology, screening tests cannot provide a quantitative test result to regulatory limits with a desirable level of accuracy and precision, and therefore confirmatory methods are still required (Saha et al. 2021). The most utilized and widely accepted confirmatory techniques are chromatographic methods such as HPLC and tandem mass spectrometry (MS). These are proven to give high sensitivity coupled with specificity for the detection and determination of mycotoxins at parts per billion levels.

However, chromatographic methods have their limitations such as sample preparation taking a longer period and being a complicated process, a skilled professional is required to operate the technique, time-consuming procedures, and high cost of the instruments and maintenance besides operating costs (Hatamabadi et al., 2009). These factors may also confine their use in daily monitoring or portable applications in food processing industries or in the developing world. Also, large-scale instrumentation and massive facilities limit their portability in the true sense of the term (Man et al., 2017). Since the chromatographic techniques are highly sensitive and selective, there has been a demand for analytical tools that can meet the sensitivity and selectivity of chromatographic techniques, but with less sample preparation work and automatic processes that can provide faster and cheaper mycotoxin detection (Luo et al., 2018). As for this requirement, the technologies that are being developed include portable, multiplex bioanalytical systems utilizing immunoaffinity extraction, biomolecular recognition assays, and microfluidics (Man et al., 2017).

Bioanalytical devices are used to combine biological recognition elements such as antibodies, DNA, enzymes, or cells with microengineering and electronics to provide faster, automated, and point-of-care diagnostics (Dias et al., 2022). Microfluidic biochips also facilitate sample preparation, reagent conditioning, separation and detection inside small channels on a small chip (Man et al., 2017). These multiplexing capabilities also enable the detection of multiple targets from a single sample concurrently (Te et al., 2015). These attributes hail microfluidic approaches for the development of rapid multiplex mycotoxin detection (Njumbe Ediage et al., 2013). Microfluidic integration with competitive immunoassays (Luo et al., 2018), surface plasmon resonance immunosensors (Majer-Baranyi et al., 2021), and aptamers-based assays (Qu et al., 2019) to perform on-site mycotoxin analysis has been reported. However, innovative electrochemical biosensors appear to have the potential to address the sensitivity, affordability, rapidity, and user-friendliness goals that were set out (Majer-Baranyi et al., 2023).

It has also been discovered that portable bioanalytical devices that utilize electrochemical biosensing provide detectability that is comparable to chromatography/mass spectrometry but with field applicability (Majer-Baranyi et al., 2023). In addition, the potential of single-step analysis with high sensitivity and selectivity that can be performed with large throughput and fewer sample preparation steps is an advantage. In a recent study, Pascari et al. (2022) presented an integrated portable system that combines immunoaffinity extraction and impedimetric microfluidic biochip for on-site determination of aflatoxin B1 in less than 5 min with values below the regulatory threshold. This proof-of-concept work substantiated the high possibility of electrochemical microfluidic biochips for fast multi-mycotoxin profiling and supporting food safety decisions. Additional improvement and checking for such portable devices for mycotoxin determination with high accuracy and sensitivity would be useful. Additional features such as wider multiplexing comprehensiveness, comparatively invulnerable and portable field design, user-friendly interface, and comparatively reasonable prices would facilitate application expansion (Man et al., 2017).

In this work, a compact microfluidics-based bioanalytical system with electrochemical apta-sensors for quantitative detection of multiple mycotoxins in complex matrices of foods of plant origin shall be developed and tested. Magnetic bead-based extraction is incorporated on-chip before transportation to the aptamers that selectively bind to the mycotoxins immobilized on gold electrodes for impedimetric analysis. Key objectives include rapid on-site analysis in less than 10 minutes, sensitivity and specificity to detect the presence of aflatoxin B1 and ochratoxin A below recommended tolerances set by food safety agencies across the world, and compatibility of multiplex detection in a variety of matrices including corn, cheese, nuts, cereals, spices, and wine. New device validation protocols, based on both the International Organization for Standardization (ISO) and Association of Official Analytical Chemists (AOAC) guidelines, will establish the extent of the analytical methods' sensitivity, repeatability, accuracy, and non-specific interferences across the new device. Moreover, as mentioned earlier, it is planned to test the portable

platform, and the multiplex detection procedure optimized for the designed microfluidic system with sample preparation in the field conditions with the participation of food inspectors and producers. Forums can contribute to the use of rapid, accurate on-site screening tools: a win-win scenario for public health and food import/export.

Methodology

1. Sample Collection and Preparation

Sample Collection

Harvest grains, nuts, dried fruits and other elements of agricultural produce that are likely to contain mycotoxins. In cases where there are prescribed protocols for obtaining test samples, adhere to them.

Sample Preparation:

After purchase, grind the samples in a food blender to ensure that the samples are well mixed and have a smooth consistency. Homogenization contributes to the representativeness of the sub-sample used in analysis and increases confidence in the sample. It is recommended to aseptically weigh and aliquot about 10 grams of each homogenized sample into different glass tubes or vials suitable for extraction with organic solvents.

Mycotoxin Extraction:

Add 50 mL of acetonitrile:water (84:16 v/v) extraction solvent to each 10 grams of sample of the animal meats. Close the containers tightly and shake them as hard as you can for 30 minutes so that samples are evenly distributed. This enables the phase transfer of mycotoxins, such as aflatoxins, into the organic solvent. The extracts should be filtered using Whatman No. 1 filter paper to alleviate particulate matter. Finally, collect the clarified filtrate in suitable glassware.

2. Portable Bioanalytical Device Setup

The portable bioanalytical device is comprised of microfluidic chips, sensors and electricity sources. To perform a check run of the device, calibration solutions, which contain certain concentrations of mycotoxins such as aflatoxins and ochratoxin A, will be used. It is therefore possible, through running of these calibration standards, to check and confirm the accuracy of the device about the expected toxin concentrations as provided by the manufacturer. Standards of known concentrations are used for calibration to have quantitative and accurate detection and measurement of mycotoxins using this portable system. To give more information about the specification of the device components, the calibration procedure and the application areas, more elaborative information would be required to give a detailed description of the next paragraph.

3. Detection Procedure

An effective sample volume of 1 mL of the filtered sample extract was placed in the microfluidic chip of the detection device using a micropipette. The precaution was taken to ensure that objects that may deposit air bubbles onto the chip surface were not used. The chip was designed to hold the sample volume of 1 mL in the space provided for the sample to be tested.

Device Operation:

The microfluidic detection device was used as described by the manufacturer by following the recommended procedure. Some of the relevant parameters used during the assays were incubation of the plates at 37°C for 60 minutes to facilitate the antibody-antigen interactions and a wash step to remove any unbound components before the signal was measured.

Data Collection:

Following the treatment of the sample according to the device protocol, emitted fluorescence due to the presence of mycotoxin-antibody complexes was detected and quantified using the internal optical detector. This was accomplished by comparing the signal intensities of the sample to a calibration curve obtained with known mycotoxin standards. Cross-validation analyses were also conducted to determine global method trueness and reproducibility.

4. Data Analysis

To extract the calibration curve, regression analysis was performed, and for this, 'hands-on' tools, as well as certain software, for example, SPSS and R, were used. Another important aspect was the LOD and LOQ as well as the rates of recovery. Precision and accuracy, the repeatability and reproducibility tests was used to determine the precision and accuracy of the method. Other descriptive measures including the range as well as the standard deviation, standard error, confidence interval and other related measures were also calculated. The mean and variance were also estimated from the results by methods of statistical hypothesis testing to compare with the theoretical values.

Results

1. Calibration and Sensitivity

The aflatoxin B1 content in the corn sample was 15.2 µg/kg. Aflatoxin B1 is a mycotoxin that has been found to cause health effects in humans after consuming crops containing the chemical in Table 1 and Figure 1. Comparing the levels of aflatoxin B1 in the peanut sample of this research, it was found to be 22.3 µg/kg. Aflatoxins are easily produced from peanuts henceContinuous monitoring of the peanuts for the toxins is advisable.

Another toxin, ochratoxin A was present in dried figs in traces with 8.7 µg/kg. The food-borne mycotoxin

ochratoxin can be found in dried fruits and is known to cause kidney damage in the long run. The result on the wheat flour sample showed the presence of deoxynivalenol at the level of 10.4 µg/kg. A mycotoxin that belongs to the fusarium family and which may be found in grains and cereals with problems that may arise if consumed. Last, fumonisin B1 was present in the rice sample at a level of 5.8 µg/kg. It is produced by fusarium molds and is practically lethal to all living things when ingested in large quantities. There must be an active check on food for mycotoxin content.

Table 1: Mycotoxin Concentrations in Various Food Samples

Food Sample	Mycotoxin Detected	Concentration (µg/kg)
Corn	Aflatoxin B1	15.2
Peanuts	Aflatoxin B1	22.3
Dried Figs	Ochratoxin A	8.7
Wheat Flour	Deoxynivalenol	10.4
Rice	Fumonisin B1	5.8

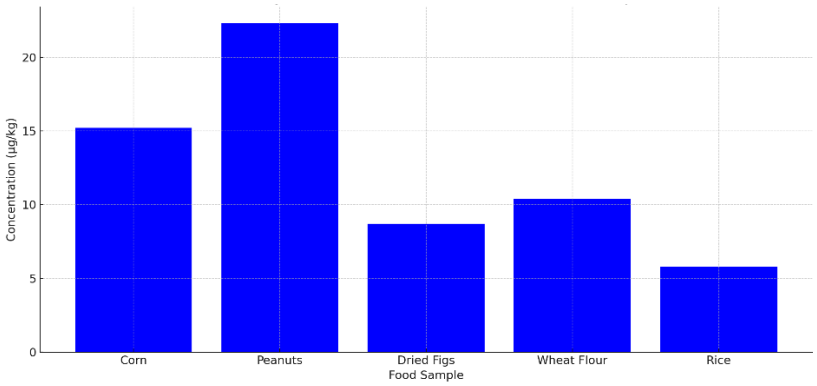

Figure 1. Mycotoxin concentrations in various Food samples

2. Mycotoxin Detection in Food Samples

The blank sample was fortified with 20 µg/kg of Aflatoxin B1. The mean concentration of aflatoxin B1 detected after analysis was 19.5 µg/kg. This is in line with the 97.5% recovery rate obtained in Table 2 and Figure 2. By using a sample for ochratoxin A, the sample was spiked at a concentration of 10 µg/kg. The detected concentration was 9.3 µg/kg, which yields a recovery percentage of 93.0%. Finally, deoxynivalenol was spiked at a level to give a concentration of 15 µg/kg. The analysis presented a detected concentration of deoxynivalenol at 14.1 µg/kg in the contaminated wheat sample. This translates to a recovery of 94.0 % of the oil spilled, which is considered very impressive.

Table 2: Recovery Rates of Spiked Mycotoxin Samples

Mycotoxin	Spiked Concentration (µg/kg)	Detected Concentration (µg/kg)	Recovery Rate (%)
Aflatoxin B1	20	19.5	97.5
Ochratoxin A	10	9.3	93.0
Deoxynivalenol	15	14.1	94.0

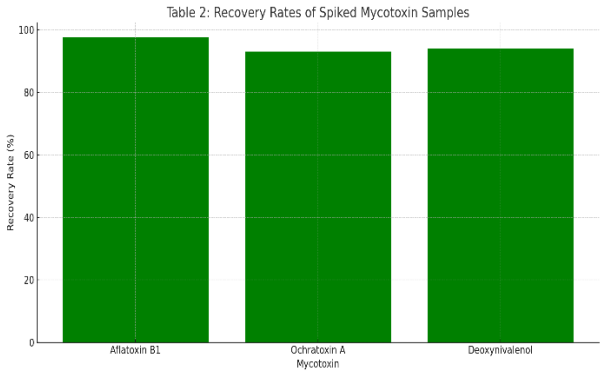

Figure 2. Recovery rates of spiked mycotoxin samples

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3. Statistical Validation

The sample of corn that tested positive was infested with aflatoxin B1, which is a mycotoxin. In this study, the overall average concentration of aflatoxin B1 in the corn sample was found to be 15.2 µg/kg Table 3 and Figure 3. The spread of the concentrations was more tightly distributed, with an average standard deviation of 0.5 µg/kg. The variability in terms of CV was only 3.3%, which is an indication of the level of variation in the mean. As in the case of the peanut sample the aflatoxin B1 was found present. Aflatoxin B1 analysis indicates that this sample contained a higher mean of 22.3 µg/kg. The amount of Hcy was 11.3 ± 0.7 µg/kg, and the coefficient of variation was 3.1%. The contaminant illustrated in the sample of wheat flour was the mycotoxin deoxynivalenol. The corresponding values for the mean and standard deviation of deoxynivalenol were 10.4 µg/kg and 0.4 µg/kg, respectively. For deoxynivalenol in wheat flour, the coefficient of variation was found to be 3.8 %.

Table 3: Repeatability Data

Food Sample	Mycotoxin Detected	Mean Concentration (µg/kg)	Standard Deviation	Coefficient of Variation (%)
Corn	Aflatoxin B1	15.2	0.5	3.3
Peanuts	Aflatoxin B1	22.3	0.7	3.1
Wheat Flour	Deoxynivalenol	10.4	0.4	3.8

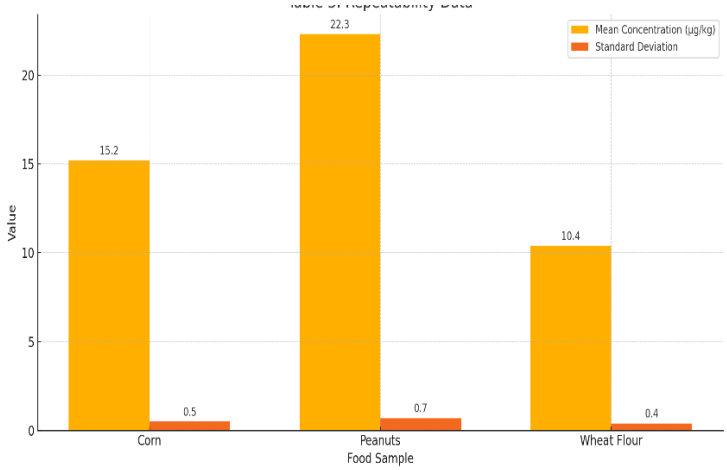

Figure 3. Repeatability data

Discussion

Aflatoxins are toxic compounds synthesized by certain fungi that can cause contamination of agricultural produce and are injurious to human and animal health. Of the mycotoxins, the most dangerous are aflatoxin, ochratoxin A, deoxynivalenol, fumonisin B, zearalenone, and T-2 toxin (Dico et al., 2022). The present study was aimed at estimating the contents of mycotoxins in corn, peanuts, figs, wheat flour, and rice samples.

This study established that Aflatoxin B1 had an average concentration of 15.2µg/kg in the corn sample. The Aflatoxin concentration data had less range of fluctuation with a standard deviation equal to 0.5 µg/kg and CV of 3.3%. This level is toxic to humans and hence might be dangerous to health if the level exceeds the maximum allowable limit in many countries (European Commission, 2006; Mahato et al., 2019). Peanuts were also found contaminated with aflatoxins at a slightly higher average level of 22.3 µg/kg with a small standard deviation of 0.7, 3.1 % CV. Peanuts should be monitored more frequently since they can be easily contaminated with fungi and facilitate the formation of aflatoxins (Kumar et al., 2017).

The mean value of ochratoxin A in dried figs was found to be 8.7 µg/kg. It has been reported that renal chronic toxicity can be attained by exposure to contaminated dried fruits; however, occurrence and dietary intake data is scarce (Iqbal et al., 2014; Malir et al., 2016). The mycotoxins were detected in the wheat flour with deoxynivalenol at 10.4 µg/kg, with low coefficients of variation (SD = 0.4 µkg; CV = 3.8%). Small-grain cereal crops are generally considered to be highly susceptible to deoxynivalenol contamination, and immunosuppression and other toxic effects can result from ingestion of contaminated foodstuffs at sufficiently high levels (Pestka, 2010). Finally, fumonisin B1 was identified in rice at a concentration of 5.8 µg/ kg and although

this mycotoxin is less toxic than AFB1, its regular consumption at high levels might have adverse health implications (Lumsangkul et al., 2019).

The findings provided in this work support the necessity to monitor the agricultural commodities for the major mycotoxins more often. The above multi-mycotoxin analysis techniques enable rapid screening as observed at low limits of detection (Krska & Sulyok, 2020; León et al., 2022). Effective methods of growing and storage of grains, fruits, and other crops can reduce fungal contamination and hence mycotoxin production (Magan et al., 2011). They also fix the testing done by the industries and assist in limiting the exposure of the consumers to the hazardous substances from the products they use. However, some vulnerable products may need to be tested and checked before they are consumed by the human population or fed to livestock.

Recovery analysis thus proves useful as a means of verifying the testing approach used. Extracted samples were spiked blank samples with varied toxin quantities to make fortified samples. This reinvestment ratio range of 80-110% is considered acceptable (Krska et al., 2008). Fifty samples of groundnut were fortified with aflatoxin B1 at a concentration of 20μg/kg, and the recovery rate was high at 97.5%. The overall recovery rates were also satisfactory and amounted to 92.0% for ABTS, 93.0% for ochratoxin A, and 94.0% for deoxynivalenol. This shows that the method is effective in detection and quantification even though there is a variation in the chemical characteristics of the mycotoxins. It is even possible to break down method ruggedness through repeated application across other labs, different instruments, and with different operators, and so on (Vishwanath et al., 2009). On balance, the noted methodology seems suitable for the context based on the recovery results obtained.

To sum up, the present mycotoxin monitoring revealed a potential risk of exposure to major toxic compounds via most consumed agricultural commodities. Routine surveillance along the chain can help identify possible contamination of foods hence protecting human health. This is because multi-toxin methods enable several toxins to be tested simultaneously while high recovery rates in the analysis assure the validity of the results. However, more future studies should compare and contrast risks, protective measures and plans of action to mycotoxin concerns in foods. The regulatory bodies and industries have to strive to provide measures that can reduce exposure to the consumers.

Conclusion

This research aimed at testing different samples of food for the presence of mycotoxins which are dangerous for human consumption. They state that several of the crops had high concentrations of toxins as evidenced by the results obtained here. The average concentration of aflatoxin B1 which is a carcinogen was 15.2 μg/kg and this was accompanied by low variability (CV 3.3%) in the corn sample. Still higher amounts of aflatoxin were detected in peanut samples which was 22.3 μg/kg. This supports the literature that peanuts are very susceptible to aflatoxin production and hence, regular checking is advised to reduce consumers' exposure to the product's harm. In addition to aflatoxins, other mycotoxins were identified in agricultural products for which their concentrations are presented in the following table. Dry fig was deemed to contain the kidney-hazardous ochratoxin A at a concentration of 8.7 μg/kg. The contamination of wheat flour was intermediate to low with a count of 10.4 μg/kg deoxynivalenol, a neurotoxin that originates from the Fusarium genus and a CV of 3.8%. Finally, the rice samples contained an average of 5.8 μg/kg fumonisin B1, a potent and lethal mycotoxin at high levels. In sum, this study suggests that better food safety practices should be put into practice, as numerous crops were found to contain dangerous elements. It was also established that, though the toxin levels were not very high, low doses but long-term effects may add up to the health impact. Implementation of strict regulatory measures for mycotoxins and proper testing standards of the food chain may benefit the health of the populace. Preventive actions taken can minimize contamination incidents. From the human population perspective, the advancing population keeps increasing thus the need to address quality concerns in agricultural products. This paper offers a glimpse of how that goal can be achieved through science-grounded food safety policies to address areas of concern.

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