Application of Raman spectroscopy in detection of Aflatoxin B1 in maize kernels

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Abstract
Use of Raman spectroscopy in detecting aflatoxin B1 (AFB1) in maize kernels is reported. Distinct difference between AFB1 contaminated and uncontaminated kernels were observed from Raman spectral profiles obtained after 532 nm excitation.

I. INTRODUCTION
Aflatoxins (AFs) are toxic and carcinogenic chemicals produced mainly by Aspergillus flavus and Aspergillus parasiticus fungi [1] growing in many agricultural products and food spices. There are four main AFs: aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2 [2]. Derivatives of the four AFs have also been reported. Aflatoxin B1 (AFB1) has been recognized as the most toxic and highly carcinogenic of all AFs [3].

Many agricultural products suffer from AFs contamination and due to the health risks posed various food safety authorities and agencies have set permissible limits of these chemicals. Farmers and agricultural products handlers or processors require a rapid and cheap detection method for these chemicals. The already developed methods including thin layer chromatography (TLC), gas chromatograph (GC), high performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), immune-affinity column assay among others. These methods are often time consuming, require trained personnel to operate equipment, and are expensive (e.g. HPLC) [4]. Here we report on use of Raman spectroscopy as a potential cheap and rapid technique in detecting AFB1 contaminated maize kernels. Recently, Lee and co-workers demonstrated its use on ground maize samples [4].

AFB1 standard in powder form obtained from Libios SARL (France) were dissolved in methanol. Various concentrations were then prepared. Maize kernel samples obtained from Kimaei Kenya seed in Kenya were randomly picked and soaked in different AFB1 concentrations. The characteristic Raman spectrum was obtained from AFB1 adsorbed on glass slides using confocal Raman spectrometer (Princeton Instruments, Acton SP2 300) equipped with two lasers emitting at 532 nm and 785 nm. The absorption spectrum was obtained from AFB1 in methanol using a UV-VIS-NIR DUV spectrophotometer (Shimadzu, Japan), for fluorescence, USB 2000 (ocean optics) spectrometer was used after excitation using an LED diode emitting at 375 nm.

II. RESULTS AND DISCUSSION
AFB1 dissolved in methanol displayed a broad absorption spectrum centered at wavelength 340 nm with bands at around 354 nm and 372 nm (see Figure 1 (a)) ascribed to vibrational transitions in the first excited singlet state of AFB1. The intensity increase with concentration reflected increase in AFB1 molecules in the solution. Fluorescence spectra peaked at around 448 nm (Figure 1 (b)) and had small maxima at 480 nm and 500 nm attributed to vibrational bands in the electronic ground state. In other studies AFB1 emission was reported to be dependent on the solvent [2, 5].

![Figure 1](image1.png)

Figure 1: Figure showing (a) absorption and (b) fluorescence spectrum of AFB1 solutions in methanol. Absorption spectrum was centered at wavelength 340 nm (Figure 1 (a)) and had small maxima at 480 nm and 500 nm attributed to vibrational bands in the electronic ground state. The intensities of both spectra increased with increase in concentration.

![Figure 2](image2.png)

Figure 2: Characteristic AFB1 spectrum together with that from glass slide substrate. Raman Signals from AFB1 were intense compared to those from glass which displayed fewer peaks thus providing little influence on AFB1 peaks.

In order to use Raman spectroscopy in detecting presence of AFB1 in maize (corn) kernels, a characteristic spectrum was first obtained. AFB1 adsorbed on a glass slide was excited using both 532 nm and 785 nm laser separately. Figure 2 shows the obtained spectra for AFB1 sample of concentration 5.0 x 10^5 ppb excited with a 532 nm laser. Spectrum obtained with 785 nm excitation (not...
AFB1 intentionally contaminated maize kernels and uncontaminated kernels were excited with a 532 nm laser and Raman scattered light detected and measured. Figure 3 displays the obtained spectra in comparison with the AFB1 characteristic Raman spectra. The contaminated maize kernels displayed profiles similar to those of AFB1 standards but with peaks displaying a 37 cm\(^{-1}\) red-shift. From this result it can be seen that Raman can be employed to detect presence of AFs in maize kernels when knowledge of their characteristic Raman spectral profiles are known. This can be a potential rapid and cheap method that can be used in the field.

III. CONCLUSIONS

The AFB1 absorption and emission spectra displayed bands ascribed to vibrational transitions. Raman spectral profiles from AFB1 adsorbed on glass slide were identical to those obtained in other studies through SERS and so showing that a glass substrate can be a cheaper alternative. Maize kernels contaminated with AFB1 exhibited Raman spectral profiles with peaks associated with AFB1 thus indicating potential use of the method in AFs detection.

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REFERENCES


