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Portable and low cost fluorescence set-up for in-situ screening of Ochratoxin A

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ABSTRACT

The present article describes a portable and low cost fluorescence set-up designed and characterized for in-situ screening of Ochratoxin A (OTA) in cocoa samples at field settings. The sensing module (the set up) consists of a LED with the wavelength of 370–380 nm and a color complementary metal oxide semiconductor (CMOS) micro-camera inbuilt at upright position of a black box to obtain an image of the sensing molecule. It allows the user to get an image of the sensing analytes under excitation conditions and process the image in order to predict the toxicity of the samples. The image capturing and processing of the system was based on the OTA concentration in the sample and analyzed data can be presented as RGB values. For each concentration of the OTA, the R, G, B co-ordinates were obtained and plotted to quantify actual OTA presents in the sample. Moreover, the system was tested for real sample analysis using cocoa contaminated with OTA. The system could detect OTA as low as 1.25 ng/ml with the maximum recovery of 87.5% in cocoa samples. The OTA was extracted in 1% $NaHCO_3$ and cleaned up using molecular imprinted polymer column (MIP). The method demonstrated a good linear range between 1.25 and 10 ng/ml. The obtained results were cross validated using chromatographic method HPLC and also compared with commercially available fluorescence instrument. The developed fluorescence setup is simple, economical, and portable with added advantages of digital image processing. The system could be deployable to cocoa fields for monitoring of OTA in quick successions. It is noteworthy to mention that this is the first report of such portable fluorescence setup where, OTA sensing was explored.

1. Introduction

Fungal toxins are frequently occurs in a wide range of food commodities such as cocoa beans [1–3]. Cocoa is predominantly produced in African countries, where the temperature is usually high and humid thereby encouraging the growth of fungal populations and subsequent production of mycotoxins. The world cocoa production is estimated at 3,592,000 t [2]. About 71% of the cocoa consumed in the world comes from Western Africa: especially from the Ivory Coast (30% of the world's production), but also from Ghana and Nigeria. Cocoa is also produced in Asia (15.8%) and Latin America (12.4%) [4]. The genera *Aspergillus* and *Penicillium* produced their metabolites such as Ochratoxin A (OTA), a common contaminant of cocoa and cocoa based products especially from these regions of Africa particularly in Nigeria [5]. However, with the maximum admissible value of OTA established in 2 μg/kg, about 40% of the cocoa which arrives in Europe may be rejected at the ports [6]. OTA was classified as a group 2B carcinogen by the International Agency for Research on Cancer (IARC) in 1993, being possibly carcinogenic to humans [7].

Cocoa is one of the major food commodities exported from Nigeria and is an important revenue source for the country with Nigeria being the fourth largest producer of cocoa in the world behind Cote d'Ivoire, Ghana and Indonesia (Cocoa growing). These raw cocoa producers are usually used in the production of other finished foods such as biscuits, coffee, chocolate, cocoa powder and sweets [8] throughout the world. Taking into account the very high consumption of chocolate and other cocoa derivatives as important ingredients in several kinds of food such as cakes, biscuits, baby foods, ice creams, sweets, etc., and in order to establish regulatory limits of OTA through different food products, data regarding OTA content in cocoa beans is needed.

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The reported methods for OTA detection in cocoa were laboratory based [9–12] and have not implemented onsite to assess the toxicity of OTA; however, there is an urgent need for cheaper, portable and field effective tools for OTA screening in cocoa beans. Recently, several methods were proposed to detect different analytes using camera based devices or field analyzers based on optical techniques at field settings [13–16]. However, still no prototype was claimed to perform the on site detection of mycotoxins.

Optical detection is one of the oldest and most established techniques [13] and has emerged as a method of choice by its simple assembly in the miniaturization of instruments for field measurements, involves the use of light emitting diode (LED) as emitter or detector. LED based systems have already been explored for sensing of different analytes (hemoglobin, O_2 and available phosphorus in soil) [14–16]. The advantages of this instrument are: functional simplicity, low-cost, low power consumption, high stability, efficiency of light production within a narrow wavelengths band, longevity [17,18] and market availability with diverse wavelengths [19]. It is easy to acquire LEDs that substitute the complex optical system obtained, when monochromatic LEDs are used as source of several wavelengths in the visible region [19,20]. Considering the use of the optical methods [21,22] with LED-based instruments has emerged as an alternative in the determination of many chemical parameters. OTA exhibits natural fluorescence at certain wavelengths, hence it becomes easy to develop sensitive detection systems using fluorescence techniques. Moreover, fluorescence has been the most commonly used form of detection in microscale biological and chemical analysis, primarily due to high sensitivity, and capable of being miniaturized into credit card size microfluidic lab-on-a-chip (LOC) systems and small portable systems [23,24].

Considering the fluorescence and the colorimetric detection in a pixel of the samples, the smallest units from which an image is composed; the pixels given to us by the display device, are measurements of the light being emitted by the sample. From these data, we can make deductions, more light may indicate the presence of a particular structure or substance, and knowing the exact value allows us to make comparisons and quantitative measurements. There are many models to work with the pixel and image processing, such as RGB (Red, Green, Blue), HSV (Hue, Saturation, Value), HIS (Hue, Saturation, Intensity), CIE L*a*b or CIExyY. The purpose of RGB color model is for the sensing, representation, and display of images in electronic systems, such as televisions or computers. This color model is a device-dependent, different devices detect or reproduce a given RGB value differently and their response to the individual R, G, and B levels. The ratio of some RGB coordinates is used for calibration purposes using digital image processing [25].

In the present work, we have designed and developed a field portable fluorescence set-up for OTA detection in cocoa based on the RGB components in the samples. The sensing module of the set up consists of a LED with the wavelength of 370–380 nm and a color CCD micro-camera inbuilt at upright position to obtain an image of the sensing liquid solution. This image is processed by the integrated microcontroller to obtain the R, G and B components of the sample. It allows the user to get a photograph of the sensing analyte under excitation conditions and process the image in order to predict the concentration (toxicity) of the samples. The developed set-up is cost effective, easy to operate and portable with added advantages of digital image processing. The advantages of the developed system over recent iphone based camera sensors are; the camera may get contaminated while analysis. Moreover, i-phone must be carried at the time of samples analysis. The system was validated with OTA, extracted from cocoa beans samples. A very good limit of detection (LOD) (1.25 ng/ml) was obtained for OTA using the developed fluorescence set up. For the extraction of OTA from cocoa, two different protocols were applied and scanned using the developed set up. It was revealed from the experimental outcome that the protocol developed by our group exhibited more fluorescence in the samples in tern more OTA in the extracted samples. Thus, further experiments were carried out using the in-house developed method for OTA detection.

2. Experimentation

2.1. Reagents and materials

Cocoa beans were provided by our industrial partners' M/s CEMOI based at Perpignan, France. All other chemicals, sodium phosphate dibasic Na_2HPO_4, potassium phosphate monobasic KH_2PO_4, sodium chloride (NaCl) ethanol (98%), HPLC-grade acetonitrile, methanol, ethanol (100%), acetic acid, tween-20 were purchased from Sigma Aldrich (France). A syringe filter, PTFE, 25 mm diameter, 0.2 μm (Scientific Strategies, USA) and syringe filter at 0.45 μm, sterile and reliable filtration were purchased from Sartorius Stedim Biotech, France. The solutions were prepared in deionized Milli-Q water (Millipore, Bedford, MA, USA). Ochratoxin A was purchased from Trilogy (France) and Ochratoxin B (OTB), derived from (*A. ochraceus*), was purchased from Santa Cruz Biotechnology, Germany. The IACs (Ochraprep®) and various syringe filters were obtained from r-biopharm AG and Sartorius, Germany respectively. The standard solutions were stored at 4 °C and OTA solutions were kept frozen (−18 °C). Molecularly imprinted solid-phase extraction columns for OTA (AFFINIMIP® SPE Ochratoxin A) were purchased from Polyintell (France).

2.2. Standard solutions

A stock standard solution of 1 mg/ml was prepared by dissolving 5 mg OTA in 5 ml of methanol and then stored at −20 °C. It has been reported that OTA solutions in methanol stored at −20 °C are stable over a period of several years [26]. Working standard solutions and calibration samples were prepared by diluting the stock solution. To prepare the phosphate buffer saline (PBS), 8 g NaCl (0.14 M NaCl), 0.2 g KCl (2.68 mM KCl), 1.15 g Na_2HPO_4 (8.1 mM Na_2HPO_4) and 0.2 g KH_2PO_4 (1.47 mM KH_2PO_4) were mixed in 1 L Milli Q ultra-pure water and pH was adjusted to 7.4.

2.3. Contamination of cocoa beans

1 kg of cocoa beans were spread on a black nylon sheet and kept under the fume hood. A 100 ml of 20, 10, 5 and 1.5 μg OTA solution was prepared in 5% ethanol and slowly sprayed over the cocoa beans. The samples were kept at room temperature for overnight.

2.4. Extraction of OTA from cocoa

2.4.1. Method a: extraction based on 1% $NaHCO_3$ in water

OTA was extracted by directly using 50 g contaminated beans with 200 ml aqueous 1% $NaHCO_3$ in a horizontal shaker for 30 min. A 50 ml aliquot was filtered using 45 μm steel filter (Fischer scientific). Before loading, the MIP column was equilibrated using 2 ml acetonitrile and then 2 ml of water. For loading, a 20 ml sample was passed through the column by keeping the flow rate constant (1 drop/s). The column was washed to remove the interferences using 8 ml 60:40 water and acetonitrile. The sample was eluted using 2 ml methanol in 2% acetic acid. The MIP columns were pre-conditioned with 10 ml PBS before sample loading.