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A method for the measurement of in line pistachio aflatoxin concentration based on the laser induced fluorescence spectroscopy

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ABSTRACT

Contamination of pistachio nuts with aflatoxin is one of the most significant issues related to pistachio health and expert. A fast pistachio aflatoxin concentration measurement method based on the laser induced fluorescence spectroscopy (LIFS) is proposed. The proposed method from theoretical and experimental points of view is analyzed. In our experiments XeCl Excimer laser is employed as an Ultra Violet (UV) source (λ=308 nm) and a UV–visible (UV–vis) spectrometer is used for fluorescent emission detection. Our setup is employed to measure the concentration of different type of Aflatoxins in pistachio nuts. Measurements results obtained by the LIFS method are compared with those are measured by the standard HPLC method. Aflatoxins concentrations are in good agreement with those are obtained by the HPLC method. The proposed laser induced fluorescence spectroscopy can be used as an in line aflatoxins concentrations measurement instrument for industrial applications.

1. Introduction

Aflatoxins are mycotoxins which can be found in a wide range of agricultural products. contamination of pistachio with Aflatoxins (AFs) Is one of the major challenges encountered by producers. among different types of Aflatoxins (AFB1, AFB2, AFG1, and AFG2), AFB1 Is the most toxic one. AFB1 Is produced by Aspergillus flavus and Aspergillus parasiticus, while AFG1 and AFG2 are products of Aspergillus parasitius [1–5]. due to High health risks associated with the presence of AFs in foods, many countries have imposed sever limits on AFs concentrations in foods. based on European COMMISSION regulation no. 165/2010, "Codex Alimentarius established a level of 15 μg/kg aflatoxin total in almonds, hazelnuts and pistachios intended for further processing and a level of 10 μg/kg aflatoxin total in almonds, hazelnuts and pistachios 'ready-to-eat and the Contam panel adopted on 16 June 2009 a statement on the effects on public health of an increase of the levels for aflatoxin total from 4 μg/kg to 10 μg/kg for tree nuts other than almonds, hazelnuts and pistachios" [6].

Detection and separation of AFs contaminated pistachios from other nuts to prevent further contamination in the beginning stage of processing is a troublesome task. An in-line, fast and accurate measurement method of AFs detection is required for pistachio producers and other nuts producers.

Thin-layer chromatography (TLC) and High Performance Liquid Chromatography (HPLC) are the most important methods for Aflatoxin detection. In fact, TLC is an official method for detection of pistachio Aflatoxin that is recommended by the association of the official analytical chemists [7–9]. Also, some researchers have used immunochemical based assays methods such as Enzyme-Linked Immune Sorbent Assay (ELISA) for AF detection in pistachios [10,11]. Although all methods based on TLC, HPLC and ELISA are quite accurate, but they are expensive, time consuming and unsuitable for in-line application. However, optical methods seem to have the potential for rapid detection of AFs contamination in pistachio contamination.

AFB1 and AFB2 emit fluorescent in bright-blue region of the spectrum (425–480 nm), and AFG1 and AFG2 emit fluorescence in blue–green range (480–500 nm) [12]. The bright greenish yellow fluorescence (BGYF) under UV-excitation has been used to detect AFs in pistachio nuts in 1980 [13]. The obtained results show a strong relationship between BGYF and AFs concentration [14]. UV lamps are used for excitation of sample and usually charged coupled device (CCD) camera is used in vision system [12].

There are many reports on attempts made to device rapid, quantitative and inexpensive fluorometric method for the purpose of measuring AFs based on the extraction of aflatoxin [15,16]. In

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agar medium in the presence of Aspergillus flavus and Aspergillus parasiticus, aflatoxin concentration variations are measured [17]. Since laser invention, many researchers have used different laser systems for spectroscopy applications [18,19]. Fluorescence spectroscopy was improved by the employment of ultraviolet lasers; this method is named laser induced fluorescence spectroscopy (LIFS) which is used in HPLC systems to enhance the sensitivity of aflatoxin measurements. This method is capable of detecting a few hundred of femtogram of each of four commonly seen aflatoxins [20]. For sensing AFB1, a system of laser induced fluorescence high performance capillary electrophoresis (LIFHPCE) was developed. Micellar electro kinetic capillary chromatography (MECC) is employed to separate AFB1, then the separated AFB1 is excited by a UV laser (375 nm) and lastly fluorescence photons (440 nm) are detected by a photo multiplier tube (PMT) [21]. Aflatoxin detection based on LIFS in mobile phase which contains an eluted sample of aflatoxins was introduced. It is shown that this method without the help of any other method can measure the sub-nanogram of aflatoxins. To measure M1 aflatoxin (AFM1) in liquids such as milk on the basis of a light emitting diode (LED) and a PMT, LIFS method was developed for industrial applications [22].

In this paper, LIFS is used to introduce an accurate and fast AFs concentration measurement method in nut shell without any manipulation or preparations before the measurement procedure. This method can be employed in an in-line industrial preprocessing.

2. Experimental setup

A scheme of experimental setup is presented in Fig. 1. As shown in this figure, a sample is illuminated by an Ultra Violet (UV) laser irradiation, and the sample fluorescence radiation is analyzed by an Ultra Violet–Visible (UV–vis) spectrometer. Selection of laser wavelength is based on the sample UV absorption spectrum while spectrometer band width must be selected based on the fluorescence emission spectrum.

The UV absorption spectrum of different types of Aflatoxin (AFB1, AFB2, AFG1 and AFG2) is presented in Fig. 2. This figure shows in 200–260 nm and near 350 nm bands are high, no matter what type of AFs is involved. Due to high absorption cross section in the first band, laser wavelength in the 200 nm region is the most suitable wavelength for AFs detection and measurement by laser fluorescence spectroscopy. Our laboratory facilities are an Excimer laser with 308 nm wavelength (Lambda physics AGX210i) and an UV–vis Spectrometer (Avantes Avaspec-2048 × 14). In our experiments, the low absorption cross section is compensated by the high power of Excimer Laser radiation. AFB1 and AFB2 fluorescence are in 420–480 nm bands while AFG1 and AFG2 are in blue–Green 480–560 nm bands. Our spectrometer is proper for detection of fluorescence emission of all type of AFs. The experimental setup is employed for AF measurement in solid pistachio. Pistachio is directly irradiated by UV laser irradiation in the sample position.

3. Sample preparation

In November 2012, some pistachio nuts were collected from Rafsanjan orchards in Iran. Samples were stored in 6 separate containers, with 150 g capacity. Some intact pistachios were kept in a container in a clean, dry and cool place, while the nut shells of some pistachios were sullied by a suspension solution of toxin including Aspergillus flavus and Aspergillu sparasiticus and placed in five other containers in laboratory desiccators. To increase humidity, some water was added in the desiccators and samples put it in sunlight were for ten days. The medium is suitable for Afs flavus growth. After 10 days, containers were moved to a refrigerator to stop the increase of contamination. The remaining four containers were moved to the refrigerator with one week interval between two successive containers. Samples were named A, B, C, D, E and F respectively. Each sample is divided into two parts, one parts was employed for the reference by the HPLC method, and the other part was used for the aflatoxin concentration measurement by the proposed laser induced fluorescence spectroscopy.

4. Experimental results and calibration

The Aspergillus flavus and Aspergillu sparasiticus were grown on the nut shell of pistachios and produced aflatoxins were diffused to the pistachio nut. The surface aflatoxin concentrations can be used as the boundary conditions for the diffusion equation and volume distribution whereas the surface distribution can be determined uniquely as a solution of diffusion equation. Hence, there is a correlation between volume and surface aflatoxin concentrations. To verify the existence of correlation and calibrate the results of the proposed measurement method, relation between the measured values and those concentrations are measured by the standard high performance liquid chromatography method are obtained experimentally. The measured values by HPLC method are criteria of the average value of aflatoxin pistachio concentration.

To measure the fluorescence spectrum of pure aflatoxins, a set of pure aflatoxin solutions with 5 μg/kg, 10 μg/kg, 15 μg/kg, and 20 μg/kg of each of the AFB1, AFB2, AFG1, and AFG2 in DI water were prepared in Rafsanjan food control laboratory. Concentrations are also verified by the standard HPLC method in our laboratory. The prepared solutions were employed for UV

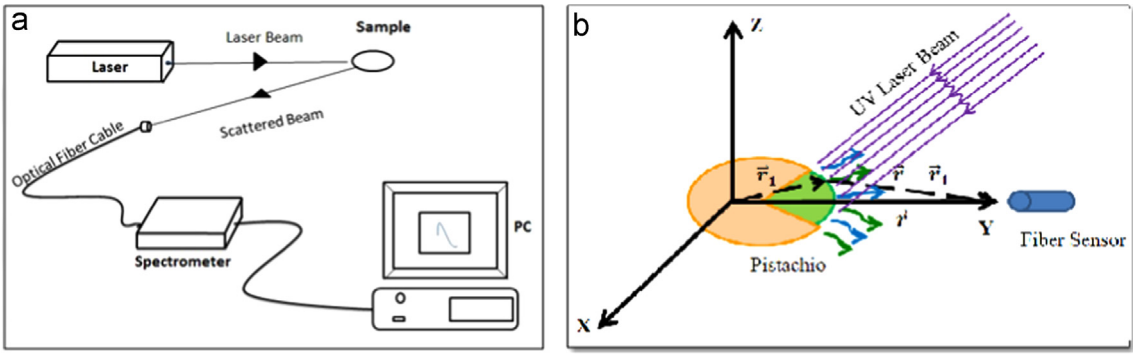

Fig. 1. (a) Schematic of experimental setup for fluorescence spectroscopy, laser is a lambda physics AGX210iXeCl (308 nm) Excimer laser, sample can be aflatoxins liquid or solid pistachio. Spectrometer is a UV–VisAvantes Avaspec-2048 × 14 spectrometer. (b) The system of coordinate for emission and detection analyses. PI is the pistachio and F is the fiber sensor.