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Application Of Surface-Enhanced Raman Spectroscopy And Chemometrics In Total Aflatoxin Detection And Quantification In An Alcoholic Beverage

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ABSTRACT
Aflatoxins (AFs) are produced by certain fungi found on agricultural crops like maize, peanuts, cottonseed, and tree nuts. The main aflatoxin producers are Aspergillus parasiticus and A. flavus. The most widely known are Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), and Aflatoxin G2 (AFG2). AFB1 is the most toxic and highly carcinogenic of all the AFs, and long-term exposure to it results in liver cancer. Thus, maximum exposure limits have been set by regulatory boards across the world. European Union (EU) and Chinese standards set the permissible AFs in human food to range between 0.004- 0.015 µg/ml and 0.005- 0.020 µg/ml respectively. The Kenya Bureau of Standard (KEBS) regulatory board set limits should not be more than 0.010 µg/ml in maize and its products. Maize and barley are susceptible to AFs contamination and are widely used as a raw ingredient in the production of commercial and traditional beers hence detection of AFs should be done on beer. Enzyme-linked Immunosorbent Assay (ELISA), High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Liquid Chromatography (LC), LC coupled mass spectrometry, and Immunoaffinity Clean-Up Columns are some of the methods used to detect AFs. These methods have some limitations as they are expensive, require specialized personnel, and involve use of chemical reagents among others. In this work, Surface Enhanced Raman Spectroscopy (SERS) is explored as a potential alternative in detecting these dangerous toxins in beer samples. This method (i.e. SERS) which is one of the most sensitive (to one molecule detection) vibrational spectroscopic technique, uses vibrational signatures of the target molecules as chemical markers. It utilizes metallic nanostructures as substrates. Here, silver nanoparticles were first synthesized by laser ablation in liquid method and characterized optically and morphologically. The Scanning Electron Microscopy (SEM) results showed that the synthesized AgNPs were spherical in shape and approximately 34 nm each in diameter. The absorbance of the localized surface plasmon (LSP) resonance band of these nanoparticles were peaked at around 404 nm and the fingerprint Raman peak centered at 214 nm. These AgNPs colloids were then mixed with clean and AF-spiked beer. The AF-spiked beer were prepared at concentrations ranging from 0.00001 µg/ml to 0.003 µg/ml (low concentrations), 0.004 µg/ml to 0.01µg/ml (permissible level range) and 0.02 µg/ml to 0.2 µg/ml (high concentrations). A drop (˜30 µl) of each of the samples were then applied, separately, onto an aluminum foil wrapped microscope glass slide and excited with a 785 nm laser when not dried and when dried and Raman spectra recorded for each sample. SERS spectral data sets were analyzed using ANOVA and PCA methods to extract the aflatoxins marker bands in beer. The bands that exhibited significant variation in intensity with aflatoxins concentration were centered at 838cm-1(ring deformation), 1016cm-1(β (C-O) and ν(C-O)), 1084cm-1(ν (CC-C) and ring deformation), 1196cm-1(β(C-H) ring β(C-H)(-CH3)), and 1386cm-1(δ(CH3)). These bands could be used as aflatoxin's Raman marker bands in beer. The bands were used as inputs to ANN models, trained and used in AFs concentration predictions. The model exhibited an accuracy of between 89-90%. The model predicted unknown concentration of Aflatoxins in beer with least concentration recorded being 0.001ug/ml and highest concentration being 0.110ug/ml. This work has demonstrated the potential use of SERS as an alternative technique to detect aflatoxins in alcoholic beverages which makes it an important tool in food industry.

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