

Elimination of Aflatoxins from Two Selected Nigerian Vegetable Oils using Magnetic Chitosan Nanoparticles

Eliminasi Aflatoksin dari Dua Minyak Nabati Nigeria Terpilih menggunakan Magnetic Chitosan Nanoparticles

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Abstract

Activated charcoal and *imarsil* (local adsorbent) had shown significant Aflatoxin (AF) decontamination potentials in vegetable oil at a low AF contamination level of ≤ 9 ng/L. AF contamination in vegetable oils can be more than a hundred-fold of this. Therefore, it is needed to investigate the potential of other adsorbents at higher AF contamination levels. Magnetic Chitosan Nanoparticle (MCNP) was synthesized, and its aflatoxins extraction efficiency from two edible vegetable oils was investigated. MCNP exhibited extraction efficiencies of 82.80 – 100% and 94.87 – 100% in palm kernel oil and palm oil at the contamination levels of 579.6 and 964.6 ng/L respectively. Total aflatoxins cleanup of the palm oil and palm kernel oil was possible at 30 °C within 30 and 60 minutes, respectively, at the optimized condition of 4.4 mg/L MCNP. MCNP concentration, temperature of extraction, and contact time were significant ($p < 0.05$) in palm kernel oil, while these conditions were not significant ($p > 0.05$) in palm oil. The results of the present investigation depict that the AF extraction efficiency of MCNP depends on the type of vegetable oil and that MCNP could be a credible alternative for AF decontamination of the investigated vegetable oil.

Keywords: aflatoxins, chitosan, contamination, nanoparticles, vegetable oils

Abstrak

Arang aktif dan *imarsil* (adsorben lokal) mempunyai potensi dekontaminasi aflatoksin (AF) yang signifikan dalam minyak nabati dengan tingkat kontaminasi AF rendah, yaitu ≤ 9 ng/L. AF dalam minyak nabati dapat lebih dari seratus kali lipat tingkat kontaminasi tersebut. Oleh karena itu, penelitian potensi adsorben lain perlu dilakukan pada tingkat kontaminasi AF yang lebih tinggi. Penelitian ini menganalisis sintesis Magnetic Chitosan Nanoparticle (MCNP) dan efisiensi ekstraksi aflatoksin dari dua minyak nabati konsumsi (minyak inti sawit dan minyak sawit). Efisiensi ekstraksi minyak inti sawit dan minyak sawit pada tingkat pencemaran 579,6 dan 964,6 ng/L, MCNP masing-masing sebesar 82,80 - 100% dan 94,87 - 100%. Pembersihan aflatoksin total pada minyak sawit dan minyak inti sawit dapat terjadi pada suhu 30 °C dalam waktu masing-masing 30 dan 60 menit, pada kondisi optimal MCNP, yaitu 4,4 mg/L. Konsentrasi MCNP, suhu ekstraksi, dan waktu kontak signifikan ($p < 0,05$) pada minyak inti sawit, tetapi kondisi ini tidak signifikan ($p > 0,05$) pada minyak kelapa sawit. Hasil penelitian ini menunjukkan bahwa efisiensi ekstraksi AF MCNP tergantung pada jenis minyak nabati dan MCNP dapat menjadi alternatif untuk dekontaminasi AF dari minyak nabati yang diteliti.

Kata kunci: aflatoksin, kitosan, kontaminasi, minyak nabati, nano partikel

INTRODUCTION

Many molds of the fungi kingdom produce secondary toxic metabolites called mycotoxins (Adeyeye, 2016; Sheikh, Levin, & Xu, 2018; Sheikh, 2019). Some of these metabolites in-

clude ergot alkaloids on cereals by *Claviceps* sp., fumonisin on maize by *Fusarium* sp., aflatoxins (AF), and ochratoxins on several plants seeds/products produced by *Aspergillus* sp. and *Penicillium* sp. (Kovač et al., 2018; Greeff-Laubacher et al., 2020). Of all the presently

known mycotoxins, AF has generated the most global concerns, perhaps due to the large volumes of foods and feeds containing them and their mutagenic and carcinogenic properties. On a global scale, AF has been detected in a significant fraction of the world's food, including maize, rice, sorghum, barley, rye, wheat, peanut, soy, cottonseed, dry fruits, and other derivative products made from these primary feedstuffs (Petrić et al., 2018). In this regard, it was estimated that more than five billion people worldwide are at risk of chronic exposure to AF through contaminated foods (Williams et al., 2004).

In the past three decades, many studies have focused on several approaches to prevent AF from entering the food chain. Some of these studies include decontamination or remediation of feed and feedstuffs (Ledoux et al., 1999), use of adsorbents such as activated charcoal and imarsil (Oluwafemi et al., 2014a), sodium and calcium aluminosilicate (Filho et al., 2016), modified rice straw (Mohamed et al., 2016), magnetic carbon nanocomposites (Zahoor & Ali Khan, 2018), activated charcoal, bentonite, and fuller's earth (Mgbeahuruike et al., 2018), and blueberry pomace bio-adsorbent (Rasheed et al., 2020).

Oil crops and their products have diverse applications in human endeavors, and this may have qualified them as the second most valuable commodity in the world trade after rice (Kolapo et al., 2012). However, it is worthy of note that there are documented reports on AF contamination of many vegetable oils obtained from their respective oil seeds. Several reports have shown high incidences of AF contamination in plant-derived oils in Ethiopia (Mohammed et al., 2016), Nigeria (Oluwafemi, Oni, & Kolapo, 2017; Oluwafemi et al., 2018; Ingenbleek et al., 2019), Benin and Mali (Ingenbleek et al., 2019). As in other foodstuffs, there are reports from outside Africa on the possibility of AF removal from peanut oil using alkaline refining process (Ji et al., 2015) and UV-irradiation (Diao et al., 2015).. However, maintaining a balance between AF removal and retention of desirable compounds, physical/organoleptic properties, and investment in process equipment has proven difficult in these processes.

A study in Africa investigated AF decontamination of vegetable oils using imarsil and activated charcoal (Oluwafemi, Oni, & Kolapo, 2017; Oni et al., 2019). In these studies, imarsil

and activated charcoal exhibited 100% adsorption efficiency within one hour at ≤ 9 ng/L AF concentration. However, at AF contamination rates of 28 - 157 ng/L, activated charcoal was not effective, while it took three hours to achieve 100% removal using imarsil as an adsorbent. However, on the understanding that AF contamination rates of 100 - 9200 ng/L have been reported in vegetable oils of Sub Sahara Africa origin (Ingenbleek et al., 2019), there is a need to investigate the decontamination efficiency of other AF adsorbent at higher contamination levels.

Given that the impact of the global AF burden is significant in low-income countries due to the lack or insufficient resources needed to tackle the aflatoxin menace; it is therefore imperative that cost-effectiveness, rapidity, safety, simplicity, and technical feasibility will determine the suitability of any AF decontamination approach in the developing countries. It is non-controversial that conventional methods of AF decontamination of food and feedstuffs are constantly improving; however current innovative nanotechnology approaches seem to suggest the use of nanoparticle adsorbents to be an effective, promising, and low-cost way to eliminate mycotoxins from foods (Xie et al., 2014; McCullum et al., 2014, Xiong et al., 2015; Horky et al., 2018). Chitosan, a natural cationic polysaccharide produced from the crustacean exoskeleton, has attracted attention in many nanotechnology applications due to its nontoxicity, biodegradability, and low immunogenicity (Horky et al., 2018). In this regard, Magnetic Chitosan Nanoparticle (MCNP) has shown to be effective, low-cost, and easily applicable nano-adsorbents (Fan et al., 2012; Zamora-Mora et al., 2014; Hosseini et al., 2016). This study investigated the efficiency of MCNP to remove aflatoxins from two widely consumed vegetable oils (palm kernel oil and palm oil) in Nigeria under different incubation conditions of contact time, dosage, temperature, and oil volume.

METHODS

Chemicals and Standards

Solvents such as acetonitrile, methylene chloride, and methanol were of High-Performance Liquid Chromatography (HPLC) grade. Aflatoxin standards were purchased from R-Biopharm (Darmstadt, Germany c/o of Chromo gen International Limited). Ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and ferrous

chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), acetic acid, ammonium hydroxide, and chitosan of high molecular weight and degree of deacetylation $\geq 75\%$ were obtained from Sigma-Aldrich Chemicals (St. Louis, MO).

Collection of Vegetable Oil Samples

The two investigated vegetable oils (palm kernel oil and palm oil) are usually refined locally before consumption in Nigeria. The low-cost grades of such oils are either unrefined or partially refined. Samples of such low-cost graded vegetable oils intended for human consumption were bought from four local markets (Ijaye, Ikorodu, Agege, and Isheri) within Lagos metropolis, Nigeria. The samples were collected into sterile amber-colored containers and transported to the laboratory for analysis. They were stored at room temperature (29 ± 2 °C) away from light until analysis.

Preparation and Characterization of Magnetic Chitosan Nanoparticles

Magnetic Fe_3O_4 -chitosan nanoparticles were prepared by chemical co-precipitation of ferrous chloride and ferric chloride with NH_3 in the presence of chitosan followed by treatment under the mechanical stirring as described earlier (Zamora-Mora et al., 2014; Hosseini et al., 2016). Iron salts in a molar ratio (2:1) (0.1622 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.0596 g $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) were dissolved in an acetic acid solution of chitosan (1 g of chitosan in 100 ml of 1% acetic acid). The resulting solution was chemically precipitated at 80 °C by adding, with mechanical stirring, a solution of 30% NH_4OH at pH 10. The dark brown product was separated by an external magnet, washed three times with distilled water, and dried under vacuum at 60 °C for 12 hours.

The size and morphology of the synthesized MCNP were obtained on Scanning Electron Microscope (SEM) Evo LS10 (Carl Zeiss Microscopy, Germany). The microscope was additionally equipped with an Energy Dispersive X-ray (EDX) system, which was used to obtain the EDX pattern of the MCNP. Fourier transform infrared spectra were obtained by a Buck Scientific, Model M530, FT-IR spectrometer.

Validation and Repeatability of Measurement

Validation of measurement was done using Wesson vegetable oil (an imported, refined vegetable oil brand) bought from the Ijaye market in Lagos, Nigeria. Seventy milliliters of Wesson

vegetable oil was weighed into a 1-liter beaker, and 450 ml of Millipore water added. The mixture was stirred vigorously with a stirrer for 5 minutes. The temperature of the oil was raised to 50 °C to ensure proper dispersion of fat. Four flasks, each containing treated Wesson oil spiked with Aflatoxin B₁ standard (0.25, 0.5, 1.0, 2.0 ng/ml), were prepared. Also, three flasks containing vegetable oils spiked with 2.0 ng/ml each of B₂, G₁ and G₂ were prepared. The aflatoxins were extracted as described below. The average recovery rates were recorded. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated using the calibration curve method.

Quantification of Aflatoxins in Vegetable Oil Samples

Vegetable oil samples were prepared for aflatoxin analysis by the modified method of Bao, Trucksess, & White (2010) and Nabizadeh et al. (2015). Fifty milliliter of the oil was measured into an Erlenmeyer flask, and 5 g of sodium chloride was added. However, for lower volumes of oil (5 - 40 ml) during the MCNP extraction experiment, proportionate sodium chloride and solvent quantity were used. One hundred and fifty milliliter of methanol : water at 80 : 20 ratio was added to the flask, and the sample was blended at high speed for 30 minutes on an orbital shaker. The homogenate was filtered using Whatman 120 mm analytical filter paper followed by filtrations with syringe filter polytetrafluoroethylene (PTFE) 0.45 μm . Filtrates of 50 ml were passed through Supelco Discovery C18 5 μm 15 x 4 mm disc (Supelco Park, Bellefonte PA) using Solid-phase Extraction (SPE). The disc was prewashed with 10 ml of distilled water twice using a disposable 25 ml syringe. Aflatoxins were slowly eluted with 0.5 ml and 0.7 ml of High-Performance Liquid Chromatography (HPLC) methanol into a 5 ml volumetric flask at a flow rate of 10 ml/minute.

The extracts were then combined and concentrated to about 0.5 ml under a gentle stream of nitrogen gas. The extract was evaporated entirely under nitrogen and then dissolved in 200 μl of the acidic 30% acetonitrile solution. A volume of 150 μl was injected into the HPLC system and analyzed for aflatoxins. The HPLC system consists of a Waters 6000A solvent delivery system and a WISP 710B sample processor for sample injections. Samples were eluted isocratically on a radically compressed 10 μm octadecyl silica

cartridge with a mobile phase of acetonitrile: methanol: water (15:15:70) at a flow rate of 2 ml/minute. A prefilter was placed between the injector and the cartridge. The aflatoxin was detected fluorometrically (excitation wavelength, 365 nm; emission wavelength 425 nm) with a fluorescence detector (model 420C). The HPLC chromatogram was recorded on a Waters data module at a chart speed of 1.0 cm/minute (Oluwafemi et al., 2014b). The concentration of aflatoxin in vegetable oil samples was determined by peak area and comparison with samples containing a known concentration of aflatoxin (spiked sample).

Decontamination of Aflatoxin Contaminated Vegetable Oils by Magnetic Chitosan Nanoparticle (MCNP)

Decontamination of naturally aflatoxin-contaminated vegetable oil was carried out using Magnetic Solid Phase Extraction of aflatoxins based on the modified method of McCullum et al. (2014). To an amber-colored Erlenmeyer flask containing 30 ml of vegetable oil, known quantity (2.2, 4.4, and 8.8 mg) of MCNP prepared above was added after weighing using Denver Instrument P-214 (Denver, Colorado). The mixture was shaken at 600 rpm for 10 minutes on an oscillator at 30 °C. The flask was placed on a magnet for 30 seconds to allow MCNP to settle down, after which the vegetable oil was decanted into another flask. The oil was taken for HPLC analysis as described previously. At the optimized quantity of 4.4 mg MCNP, decontamination of contaminated oils under different incubation conditions of the volume of oil (5, 20, and 40 ml), contact time (30, 60, and 120 minutes), and temperature of oil (30, 70 and 100 °C) were carried out. The percentage reduction of aflatoxins (B1, B2, G1, and G2) in the investigated oil samples by using MCNP was subsequently calculated by comparing the aflatoxin concentration of the oil before and after decontamination.

Statistical Analysis

Data obtained were expressed as means \pm standard deviation. Analysis of variance was carried out on the data obtained to determine the significance of differences. A two-tailed p-value of less than 0.05 was considered to be statistically significant. Values that were significantly different were separated using the Duncan

Multiple Range test using SPSS for Windows Version 17.0 statistical package.

RESULTS AND ANALYSIS

Figure 1 shows the scanning electron microscopy image of the magnetic chitosan nanoparticles at a magnification of 50,000 X. The particles were found to be poly-dispersed and have 250 – 400 nm of average size. The particles seem to be scattered without aggregation. The image shows that the magnetic nanoparticles were encapsulated evenly in the cross-linked chitosan adsorbents. The MCNP size range in the present study is much higher than the value of 30 - 100 nm reported for magnetic chitosan nanoparticles grafted with β -cyclodextrin (Karimnezhad & Moghimi, 2014).

The spot profile EDX spectrum of MCNP (Figure 2) indicates the composition of the synthesized magnetic nanoparticles. The spectra show strong peaks of Fe, which confirm the presence of iron in the nanohybrid with 54.35% weight. This result demonstrates the high purity of the magnetite nanoparticles. The presence of oxygen and carbon peaks resulted from chitosan employed in the nanohybrid synthesis, and the presence of other elements such as potassium can be a result of impurities. This observation is also supported by the shift and absorption peaks observed in the Fourier Transform Infrared Spectroscopy (FT-IR) analysis.

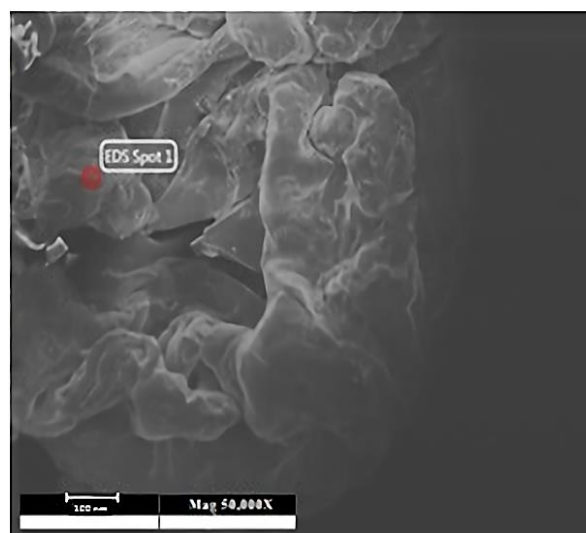


Figure 1. Scanning Electron Micrograph of Magnetic Chitosan Nanoparticle at a Magnification of 50,000 X

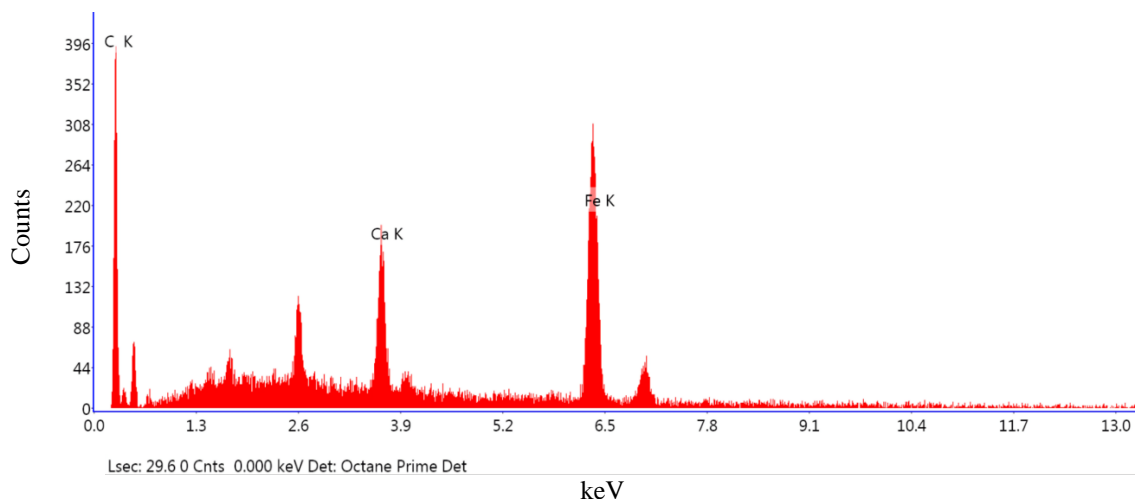


Figure 2. Energy Dispersive X- Ray Spectrum of Magnetic Chitosan Nanoparticle

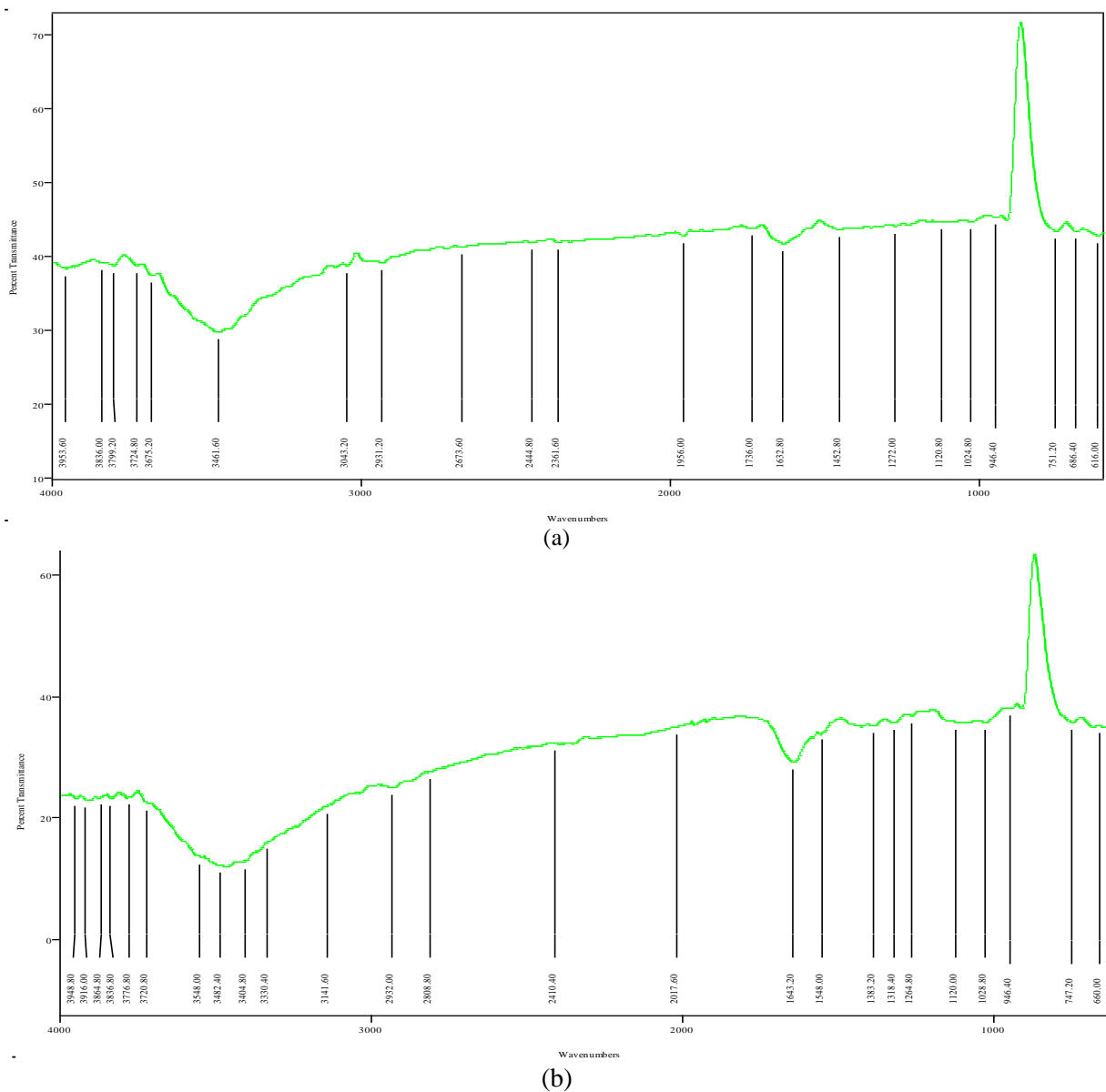


Figure 3. Infra-Red Spectrum of Fe²⁺- Fe³⁺- Chitosan Nanohybrid (a) and Chitosan (b)

Fourier Transform Infrared Spectrophotometer (FT-IR) is the most essential and powerful tool for identifying the functional groups present in the sample. The wavelength of light absorbed is the characteristic of the chemical bond. The chemical bonds in a molecule can be determined by interpreting the infrared absorption spectrum (Naresh et al., 2018). Figure 3 depicts the FT-IR spectra of the Fe²⁺ and Fe³⁺ interaction with chitosan to form nanohybrid (a) and chitosan (b). In the FT-IR spectra (Figure 3a), there was a strong absorption band around 3461 cm⁻¹, which represents N-H stretching vibrations, cha-

racteristic of the presence of amino acids. The absorption band around 1632 cm⁻¹ with low intensity can be assigned to N-H bending of amine, which confirms the presence of N-H of amine. The bands at 686 cm⁻¹ and 751 cm⁻¹ can be attributed to Fe-O stretching of deposited magnetite in the carbon nanostructure. It was observed that there was a change in absorption band value of the chitosan compared to the nanohybrid formed, which confirms the interaction of chitosan with Fe²⁺ and Fe³⁺ to form nanohybrid, indicating that chitosan was successfully coated onto the surfaces of the magnetic nanoparticles.

Table 1. Aflatoxins level in palm kernel oil treated with magnetic chitosan nanoparticle (MCNP) under different incubation conditions

	Incubation condition	AFB1 (µg/L)	AFB2 (µg/L)	AFG1 (µg/L)	AFG2 (µg/L)	TOTAL (µg/L)	Aflatoxin Reduction (%)
Before MCNP Treatment		0.41620 ±0.0009	0.0048 ±0.0001	0.0524 ±0.0002	0.1062 ±0.0004	0.5796 ±0.0003	
After Decontamination	MCNP Quantity						
	2.2 mg	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0943 ±0.0001 ^c	0.0000 ±0.0000 ^a	0.0943 ±0.0001 ^c	83.73 ^a
	4.4 mg	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0321 ±0.0002 ^b	0.0000 ±0.0000 ^a	0.0321 ±0.0002 ^b	94.46 ^b
	8.8 mg	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0136 ±0.0004 ^a	0.0024 ±0.0001 ^b	0.0160 ±0.0002 ^a	97.24 ^b
	Volume of oil (@ 4.4 mg MCNP)						
	5 ml	0.0202 ±0.0003 ^c	0.0000 ±0.0000 ^a	0.0713 ±0.0002 ^b	0.0032 ±0.0000 ^b	0.0947 ±0.0002 ^b	83.66 ^a
	20 ml	0.0158 ±0.0003 ^b	0.0010 ±0.0000 ^c	0.0797 ±0.0003 ^b	0.0032 ±0.0000 ^b	0.0997 ±0.0003 ^c	82.80 ^a
	40 ml	0.0123 ±0.0002 ^a	0.0002 ±0.0000 ^b	0.0565 ±0.0003 ^a	0.0024 ±0.0000 ^a	0.0714 ±0.0002 ^a	87.68 ^a
	Temperature (@ 4.4mg MCNP)						
	30 °C	0.0168 ±0.0004 ^b	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0168 ±0.0004 ^b	97.10 ^a
	70 °C	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	100 ^b
	100 °C	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	100 ^b
	Contact time (@ 4.4mg MCNP)						
30 minutes	0.0228 ±0.0004 ^b	0.0008 ±0.0000 ^b	0.0517 ±0.0005 ^b	0.0000 ±0.0000 ^a	0.0753 ±0.0004 ^b	87.01 ^a	
60 minutes	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	100 ^c	
120 minutes	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	0.0000 ±0.0000 ^a	100 ^c	

Values are means ± standard deviation (n=3). Within column of each treatment values with different superscripts are significantly different (p < 0.05).

Aflatoxin recoveries from spiked vegetable oil for all four analogs ranged 98 - 100% over the four concentrations. The calibration curves showed good linearity. The LOD values of AFB1, B2, G1, and G2 were 0.422 ng/L, 0.292 ng/L, 0.0028 ng/L, and 0.185 ng/L respectively. The LOQ values of AFB1, B2, G1, and G2 were 1.266 ng/L, 0.873 ng/L, 0.0085 ng/L, and 0.555 ng/L respectively. Table 1 and Table 2 show the results of aflatoxin decontamination of contaminated palm kernel oil and palm oil using the synthesized MCNP adsorbent under different incubation conditions. Before decontamination, the AF contents of palm kernel oil and palm oil were

0.5796 and 0.9646 $\mu\text{g/L}$, respectively. These values are comparable to the range reported for palm oil (0.2 - 5.3 $\mu\text{g/kg}$) and peanut oil (0.5 - 8.7 $\mu\text{g/kg}$) in Nigeria; peanut oil (0.4 - 8.3 $\mu\text{g/kg}$) in Benin; other vegetable oil (0.1 - 9.2 $\mu\text{g/kg}$) in Cameroon (Ingenbleek et al., 2019). An earlier study in Nigeria reported the AF contents of 157, 49, 33, 28, 9, 5, and 4 ng/kg in corn oil, coconut oil, olive oil, soya oil, palm kernel oil, palm oil, and groundnut oil, respectively (Oluwafemi, Oni, & Kolapo, 2017). In Haiti, Schwartzbord & Brown (2015) reported the presence of AF in peanut oil at the level of 19 - 185 $\mu\text{g/kg}$.

Table 2 Aflatoxins level in palm oil treated with magnetic chitosan nanoparticle (MCNP) under different incubation conditions

	Incubation Condition	AFB1 ($\mu\text{g/L}$)	AFB2 ($\mu\text{g/L}$)	AFG1 ($\mu\text{g/L}$)	AFG2 ($\mu\text{g/L}$)	TOTAL ($\mu\text{g/L}$)	Aflatoxin Reduction (%)
	Before MCNP Treatment	0.0491 ± 0.0007	0.0433 ± 0.0006	0.6966 ± 0.0010	0.1756 ± 0.0005	0.9646 ± 0.0007	
After decontamination	MCNP Quantity						
	2.2 mg	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	100 ^a
	4.4 mg	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	100 ^a
	8.8 mg	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	100 ^a
	Volume of oil (@ 4.4 mg MCNP)						
	5 ml	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	100 ^b
	20 ml	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0495 $\pm 0.0003^c$	0.0000 $\pm 0.0000^a$	0.0495 $\pm 0.0003^c$	94.87 ^a
	40 ml	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0054 $\pm 0.0004^b$	0.0000 $\pm 0.0000^a$	0.0054 $\pm 0.0004^b$	99.44 ^b
	Temperature (@ 4.4 mg MCNP)						
	30 °C	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0023 $\pm 0.0001^b$	0.0023 $\pm 0.0001^b$	99.76 ^a
	70 °C	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	100 ^a
	100 °C	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	100 ^a
Contact time (@ 4.4 mg MCNP)							
30 minutes	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	100 ^a	
60 minutes	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	100 ^a	
120 minutes	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	0.0000 $\pm 0.0000^a$	100 ^a	

Values are means \pm standard deviation (n = 3). Within column of each treatment values with different superscripts are significantly different (p < 0.05).

Meanwhile, Shephard (2018) noted that data on the occurrence of AF in vegetable oil the world over indicates that crude or unrefined artisanal oils in the developing marketplace cause concern. The AF content values obtained in the present study are lower than the EU (4 µg/kg) and Codex (15 µg/kg) standards (Codex Alimentarius Commission, 1995; The Commission of The European Communities, 2006). However, understanding that the vegetable oils investigated in this study are often consumed unrefined in Nigeria and used in many food applications daily, especially by the poor majority, generates a significant concern as the consuming populace stands a great chance of chronic AF exposures. Therefore, there is an urgent need to protect them from impending future aflatoxicosis.

In this study, the use of MCNP resulted in AF decontamination efficiencies of 82.80–100% and 94.87–100% in palm kernel oil and palm oil, respectively. In palm kernel oil, MCNP concentration, the temperature of decontamination, and contact time significantly ($p < 0.05$) determined the extent of observed decontamination. However, these incubation conditions had no significant ($p > 0.05$) effect on the observed AF removal efficiencies in palm oil. Furthermore, total AF cleanup of the palm oil and palm kernel oil was possible within 30 and 60 minutes respectively at the optimized condition of 4.4 mg MCNP concentration. Generally, the results of the present investigation depict that the AF decontamination efficiency of MCNP depends on vegetable oil type, with palm oil more easily amenable to decontamination than Palm kernel oil.

Chitosan is a highly functionalized polysaccharide containing hydroxyl groups, acetyl amine, or free amino groups. This property has contributed immensely to its usage as an adsorbent in many nano-applications. In this regard, Luo, Zhou, & Yue (2017) reported on high magnetic properties and patulin adsorption capabilities of magnetic Fe₃O₄ chitosan nanoparticles. The high adsorptive capabilities of chitosan nanoparticles have been attributed to hydrogen bonding formation between the adsorbates and multiple functional groups, including hydroxyl groups, acetyl amine, or free amino groups (Fan et al., 2012; Hosseini et al., 2016). In the present study, the high AF removal capacity of MCNP might be attributed to hydrogen bonding between AF contaminants in the oil and multiple function-

al groups present on the MCNP. Giakoumis (2013) reported that palm oil has a higher degree of unsaturation (hence greater fluidity) than palm kernel oil. The higher degree of decontamination observed in palm oil in the present study might be suggesting that AF removal by the MCNP might be positively correlated with the degree of fluidity of the investigated oil.

CONCLUSIONS

Data from the present study indicate that MCNP can carry out total AF decontamination of palm kernel oil and palm oil at AF concentrations of 0.5796 and 0.964 µg/L within 60 and 30 minutes respectively at tropical room temperature of 30 °C. In this regard, palm oil is more amenable to decontamination compared to palm kernel oil. The present study results indicate an improvement over the earlier reported attempts using activated charcoal and imarsil. The observed decontamination efficacy at room temperature will reduce the energy cost of the decontamination operation. The present investigation indicates that MCNP could be a credible alternative for AF decontamination of palm oil and palm kernel oil. Therefore, further study on the AF decontamination efficacy of MCNP at higher AF contamination levels and vegetable oil volume should be undertaken.

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