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DECONTAMINATION OF AFLATOXINS IN VEGETABLE OILS USING ACTIVATED CHARCOAL AND IMARSIL®

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ABSTRACT

Infestation of vegetable oils by mycotoxins affects its quality and general acceptability thereby resulting in huge economic losses in addition to being hazardous to consumer health. To screen, and remove aflatoxins (AF) in vegetable oils using inexpensive, readily available adsorbents [imarsil® and activated charcoal (AC)], edible commercial vegetable oils from ten plant sources (cotton, sunflower, canola, palm-kernel, olive, groundnut, soya-bean, coconut, palm and corn) obtained from Nigerian markets were assessed for aflatoxin (AF) levels using High Performance Liquid Chromatography (HPLC) method. Adsorption studies of AF were performed on the AF positive oils using imarsil® and activated charcoal (AC) at 2 and 3% concentrations under 1 and 3 hours contact time. AF concentrations were: corn oil 157 µg/kg, coconut oil 49 µg/kg, olive oil 33 µg/kg, soya oil 28 µg/kg, palm kernel oil 9 µg/kg, palm oil 5 µg/kg and groundnut oil 4 µg/kg, while cotton, sunflower and canola oils had no detectable AF levels. At ≤ 9 µg/kg AF contamination rate, both imarsil® and AC exhibited 100% adsorption efficiency within one hour. At AF contamination rates of 28-157 µg/kg, AC was not effective while imarsil® had 100% removal efficiency in 3 hours. The study therefore revealed that incorporating imarsil® into the oil refining process could help in the management of aflatoxicosis globally.

Keywords: Activated charcoal, Adsorbent, Aflatoxicosis, Imarsil®, Inexpensive, Refining

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INTRODUCTION

Fats and oils are crucial part of our dietary composition, supplying nutrients, aiding in the absorption of vitamins, and providing energy for our body. They are the main dietary sources of vitamin E and are particularly important for bone, joint and skin (Rader *et al*., 2007). The health benefits of using edible vegetable oils have boosted their demand and consumption worldwide, especially in the developed countries (Karunarathna *et al*., 2019). The demand for vegetable oil cuts across domestic cooking, and its utilization as an ingredient for other

food productions (in baked goods and fried snack foods). Vegetable oil is extracted primarily from seeds of oilseed plants and those commonly used worldwide include; corn, soybean, olive, cotton, palm, palm kernel, and groundnut (Behrman and Venkat, 2005).

Aflatoxin contamination in crop is conceived along laterally with the food chain during plant development, harvesting, storage and processing (Jallow *et al*., 2021). According to Eshola *et al*., (2020), 25% of the world's agricultural produce are affected by mycotoxins and the most potent and thoroughly studied of the mycotoxins are aflatoxins. Mycotoxins are more prevalent in tropical and subtropical areas where environmental conditions such as high temperature and humidity prevail, favouring the growth of fungi, and production of mycotoxins on the crops. The four major types of aflatoxins are B1, B2, G1, G2 while M1 and M2 are metabolites of B1 and B2 respectively (Iqbal *et al*., 2012). Over the years contamination of food with aflatoxins (AFs) has been a global food safety concern (Karunarathna *et al*., 2019).

Aflatoxigenic fungi are found in different food commodities including nuts, oils and cereals (Pitt and Hocking, 2009). They may contaminate foods by colonizing them at several stages of the food chain; harvesting, processing, transportation and storage (Manonmani *et al*., 2005).
Previous studies have established the presence of Aflatoxins (AF) in many oil seeds (Mariod and Idris, 2015; Razis *et al*., 2020). from which most commercial vegetable oils are extracted. These oil seeds are used for production of vegetable oils. It is therefore highly probable that such vegetable oils will be contaminated.

Imarsil® has been reported as an inexpensive and locally-sourced synthetic adsorbent obtained from oxidized natural polymer of *Brachystegia nigerica*. It has been reported to be highly potent in removing AF from cow milk (Oluwafemi *et al.,* 2014a). In some eastern states of Nigeria, *B. nigerica* (*Leguminosae*) is used as condiment to thicken soup due to its hydrocolloid or gelling property (Okwu, 2004). As an oxidized polymer of *B. nigerica*, it is considered as an efficient adsorbent because of its quick and simple recovery approach especially in the clarification of microbial enzymes from fermentation broth (Akpan and Kareem, 2002).

Activated charcoal (AC), is a non-specific adsorbent, and therefore, will bind based on relative concentration of nutrients or compounds in the feed. Carbon source, preparation methods, pore size distribution and surface area are factors that determine the degree to which an AC will bind the mycotoxin (Doll *et al*., 2004). Several studies have reported variable results in the ability of AC to bind aflatoxin *in vitro* and *in vivo*, AC may be important in binding zearalenone and/or deoxynivalenol (Bueno *et al*., 2005). In an *in vitro* gastrointestinal model, AC reduced availability of deoxynivalenol and nivalenol (Avantaggiato *et al*., 2004). In a previous study, Oluwafemi *et al.,* (2018), reported the extent of microbial contamination of refined, and unrefined vegetable oils sold in South-West Nigeria. The current study was aimed at evaluating the potential of imarsil® and AC on aflatoxin detoxification in selected edible oils sold in South West Nigeria by quantifying aflatoxin levels in the oils before, and after treatment with imarsil® and AC respectively.

MATERIALS AND METHODS

Chemicals and Standards

Imarsil® was prepared as described by Akpan and Kareem (2002). Under Nigerian patent No RP14784.

The aflatoxin standard was purchased from Chromogen (New Delhi, Delhi, India). Immunoaffinity column was Aflastar™ R, supplied by Roma Labs Diagnostic Technopark 13430, Tulln, Austria. Solvents such as acetonitrile, methylene chloride, and methanol were of HPLC grade.

Activated charcoal (AC) or charcoal, is a very porous, non-soluble powder formed by the pyrolysis of organic materials. Activated charcoal was purchased from Shoprite Store Dugbe, Ibadan, Oyo State.

Collection of vegetable oil samples

Vegetable oils (both local and imported) of different plant sources (sunflower, cotton, canola, olive, palm-kernel, groundnut, coconut, soya-beans, corn, and palm) were purchased from open markets in Abeokuta, Ogun State, Nigeria. The samples were collected into sterile containers and transported to the laboratory for analysis.

Aflatoxin quantification of vegetable oil samples

Aflatoxin quantification in the vegetable oil samples was carried out in duplicates according to the modified method of Rajarajan *et al*., (2013). Fifty milliliters of the oil was measured into erlenmeyer flask, and 5 g of sodium chloride added. 100 ml 80% methanol: water in the ratio 80:20 was added to the flask, and the sample was blended at 200 rpm for 30 min on an orbital shaker. Absorption was detected by fluorescence detection with excitation λ365 nm and emission λ464 nm.

Control Studies

Detoxification studies of aflatoxin was done using a 2 × 2 × 2 factorial design involving two contact times (1 and 3 h), two adsorbents (activated charcoal and imarsil®) and two concentrations of the adsorbents (2 and 3%). Vegetable oils samples that were positive for aflatoxin were passed through a separating funnel bedded with dietary activated charcoal and imarsil® at concentrations of 2 and 3 % according to the method described by Oluwafemi *et al.,* (2014b). The experimental setups were in place for 3 h. Samples were taken at 1 and 3 h. The experiment was carried out at room temperature (28±2 ºC).

Statistical Analysis

Data obtained were subjected to statistical analyses using the Statistical Package for Social Sciences (SPSS) version 20.0 (IBM, 2011). Mean values were compared using one-way analysis of variance (ANOVA). Results were presented as Mean±Standard deviation. Post hoc test was done using the Student-Newman-Keuls (SNK). A P – value of less than 0.05 was considered to be statistically significant.

RESULTS

Aflatoxins were not detected in the cotton oil, sunflower oil and canola oil samples evaluated in this study (Table 1). Similarly, only Aflatoxin B1 was recorded in the corn oil and palm kernel oil samples. Also, only AFB2 was recorded in the palm oil and groundnut oil samples. However, levels of AFB1 and total aflatoxins were significantly higher in the corn oil than those of the other oils. AFB2 was only observed in olive oil, palm oil and ground-nut oil samples and these were not significantly different. Aflatoxin G1 was recorded in olive oil samples. Aflatoxin G2 was recorded in coconut oil and soya oil samples and was highest in the coconut oil

sample.

Corn oil with 157.0 μg/kg aflatoxin at 2% concentration after 1h gave a significant reduction to 72.00 μg/kg and at 3h gave 31.00 μg/kg (Table 2). Coconut oil with 49.00 μg/kg aflatoxin at 2% concentration after 1h gave a reduction to 18.00 μg/kg and at 3h gave 0.000 μg/kg. Olive oil with 33.00 μg/kg aflatoxin at 2% concentration after 1h gave a significant reduction to 14.00 μg/kg and at 3h gave 0.000 μg/kg. Soya oil with 28.00 μg/kg aflatoxin at 2% concentration after 1h gave a reduction to 1.000 μg/kg 1 and at 3h gave 0.000 μg/kg. Soya, palm kernel, groundnut and palm oil also gave reduced values after adsorption (Table 2).

For the corn oil treated with 2% imarsil:, after 1 hour of treatment, the aflatoxin concentration in corn oil was reduced by 72% and after 3 hours of treatment, the aflatoxin concentration was reduced by 31% (Table 3). In contrast, when using 3% imarsil for 3 hours, the aflatoxin concentration in corn oil was reduced to 0% (Table 3). Similar observations were also made for other treatment combinations and oil types.

Table 1: Aflatoxin levels (μg/kg) in edible vegetable oils sold in Abeokuta, Nigeria

Sample	AFB_1	AFB_2	AFG_1	AFG_2	≤Total AF
Cotton oil	$0.00.00\pm0.00^{d}$	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.000^{e}
Sunflower oil	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.000^{e}
Canola oil	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.000^{e}
Corn oil	$157.0.00\pm0.58^{a}$	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	157.000^{a}
Coconut oil	16.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	33.00 ± 0.58^{a}	49.000^{b}
Olive oil	7.00 ± 0.58^{c}	3.00 ± 0.58^{a}	23.00 ± 0.58^{a}	0.00 ± 0.00^{c}	33.000^{c}
Soya oil	7.00 ± 0.58^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	21.00 ± 0.58^{b}	28.000^{c}
Palm Kernel oil	9.00 ± 0.58^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	9.000^{d}
Palm oil	0.00 ± 0.00^{d}	5.00 ± 0.58^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	5.000^{d}
Ground-nut oil	0.00 ± 0.00^{d}	4.00 ± 0.58^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	4.000^{d}

^{abcde}Mean (±Standard deviation) in the same column having similar superscripts are not significantly different ($p \leq 0.05$)

Table 2: Reduction of aflatoxin concentrations (µg/kg) in vegetable oils using *imarsil*® and activated charcoal

Veg. Oil	Initial concentrations of AF and Treatment with absorbents	Concentrations after Contact times (Hour) with Adsorbent	
		1	3
Corn Oil	157 µg/kg AF + 2 % imarsil	71.67±0.58^{c}	31.00±0.00^{c}
	157 µg/kg AF + 3 % imarsil	48.00±0.00^{d}	0.00±0.00^{d}
	157 µg/kg AF + 2 % activ.charcoal	121.67±0.58^{a}	91.67±0.58^{a}
	157 µg/kg AF + 3 % activ.charcoal	101.67±0.58^{b}	87.33±0.58^{b}
Coconut oil	49 µg/kg AF + 2 % imarsil	17.67±0.58^{c}	0.00±0.00^{c}
	49 µg/kg AF + 3 % imarsil	0.00±0.00^{d}	0.00±0.00^{c}
	49 µg/kg AF + 2 % activ. Charcoal	42.67±0.58^{a}	34.67±0.58^{a}
	49 µg/kg AF + 3 % activ.charcoal	38.33±0.58^{b}	24.00±0.00^{b}
Olive oil	33 µg/kg AF + 2 % imarsil	14.00±0.00^{b}	0.00±0.00^{b}
	33 µg/kg AF + 3 % imarsil	0.00±0.00^{c}	0.00±0.00^{b}
	33 µg/kg AF + 2 % activ. Charcoal	29.00±0.00^{a}	21.67±0.58^{a}
	33 µg/kg AF + 3 % activ. Charcoal	27.17±0.29^{a}	21.00±0.00^{a}
Soya oil	28 µg/kg AF + 2 % imarsil	10.67±0.58^{b}	0.00±0.00^{b}
	28 µg/kg AF + 3 % imarsil	0.00±0.00^{c}	0.00±0.00^{b}
	28 µg/kg AF + 2 % activ. Charcoal	21.83±0.29^{a}	17.00±0.00^{a}
	28 µg/kg AF + 3 % activ. Charcoal	21.00±0.00^{a}	15.00±0.00^{a}
Palm kernel oil	9 µg/kg AF + 2 % imarsil	0.00±0.00^{b}	0.00±0.00^{b}
	9 µg/kg AF + 3 % imarsil	0.00±0.00^{b}	0.00±0.00^{b}
	9 µg/kg AF + 2 % activ. Charcoal	5.00±0.00^{a}	0.67±0.58^{a}
	9 µg/kg AF + 3 % active. Charcoal	4.00±0.00^{a}	0.00±0.00^{b}
Palm oil	5 µg/kg AF + 2 % imarsil	0.00±0.00^{b}	0.00±0.00^{a}
	5 µg/kg AF + 3 % imarsil	0.00±0.00^{b}	0.00±0.00^{a}
	5 µg/kg AF + 2 % activ. Charcoal	2.00±0.00^{a}	0.00±0.00^{a}
	5 µg/kg AF + 3 % activ. Charcoal	1.00±0.00^{a}	0.00±0.00^{a}
Groundnut. Oil	4 µg/kg AF + 2 % imarsil	0.00±0.00^{a}	0.00±0.00^{a}
	4 µg/kg AF + 3 % imarsil	0.00±0.00^{a}	0.00±0.00^{a}
	4 µg/kg AF + 2 % activ. Charcoal	0.00±0.00^{a}	0.00±0.00^{a}
	4 µg/kg AF + 3 % activ. Charcoal	0.00±0.00^{a}	0.00±0.00^{a}

^{abcd}Mean (±Standard deviation) in the same column for each of the vegetable oil having similar superscripts are not significantly different ($p \leq 0.05$)

Tables 3: Decontamination efficiency (%) in residual concentrations percentage of aflatoxins (µg/kg) in vegetable oils using *imarsil*® and activated charcoal

Veg. Oil	Treatment	Contact time 1 hr (%)	Contact time 3 hr (%)
Corn Oil	2 % imarsil	72	31
	3 % imarsil	48	0
	2 % activ.charcoal	122	92
	3 % activ.charcoal	102	87
Coconut oil	2 % imarsil	18	0
	3 % imarsil	0	0
	2 % activ. Charcoal	43	35
	3 % activ. Charcoal	38	24
Olive oil	2 % imarsil	14	0
	3 % imarsil	0	0
	2 % activ. Charcoal	29	22
	3 % activ. Charcoal	27	21
Soya oil	2 % imarsil	11	0
	3 % imarsil	0	0
	2 % activ. Charcoal	22	17
	3 % activ. Charcoal	21	15
Palm kernel oil	2 % imarsil	0	0
	3 % imarsil	0	0
	2 % activ. Charcoal	5	1
	3 % activ. Charcoal	4	0
Palm oil	2 % imarsil	0	0
	3 % imarsil	0	0
	2 % activ. Charcoal	2	0
	3 % activ. Charcoal	1	0
Groundnut. Oil	2 % imarsil	0	0
	3 % imarsil	0	0
	2 % activ. Charcoal	0	0
	3 % activ. Charcoal	0	0

DISCUSSION

The highest aflatoxin level was from corn oil. This agrees with the findings of the International Agency for Cancer Research, (IARC, 2002), that maize oil seed, and most crops are frequently contaminated with aflatoxins. Aflatoxin B1 was the most predominant in corn oil. IARC, (2004), advocated for a dietary diversification from maize to other cereals like sorghum or millet due to the adverse health effects of aflatoxin exposure. Oni *et al.,* (2022), recently opined that drying of maize to safe moisture level could help reduce aflatoxin proliferation in this product, so proper and efficient drying method of maize should be ensured to mitigate against the deleterious effects on the different end products such as cereals, oils etc. Exposures to aflatoxins in Nigeria through consumption of contaminated foods have been reported by Oluwafemi *et al.,* (2014 a, b). The International Agency for

Cancer Research, (IARC, 2004) estimated that the aflatoxin exposure in Nigeria is high, ranging from 139 to 227 μg/kg body weight. This value was estimated from maize consumption pattern in Nigeria.

Aflatoxin B1 was the most prevalent of all aflatoxins in the positive oil samples while Aflatoxin G1 was not found in several of the oil samples, this is in agreement with the findings of Oluwafemi *et al.,* (2014a) who reported that amongst all fractions, AFB1 is normally the most predominant in food, and feed products in Nigeria. The absence of AFG in the same samples could be explained by the fact that several *Aspergillus* spp. isolates do not produce AFG (Alam *et al*., 2010). The Joint FAO/WHO Expert Committee on Food Additives (EC 2010), established a tolerable daily intake (TDI), but strongly recommended that the level of aflatoxin should be as low as possible. Low level of aflatoxins approved for refined oils according to (EU 2010), is 50 μg/kg. The only sample that exceeded this regulatory limit is the corn oil sample.

At aflatoxin contamination rate of 9 μg/kg and below, imarsil® and AC exhibited 100 % adsorption efficiency, within one hour of treatment. However, at higher AF contaminations, of 28 - 157 μg/kg, AC was not as effective as 3 % concentration, imarsil® resulted in 100 % adsorption within 3 hours contact time while 28.6 and 51.0 % were obtained for AC within the same time. The potency of AC was significantly ($p<0.05$) lower than the observed values for imarsil® and this agrees with Akpan and Kareem (2002), in the use of imarsil® for enzyme purification. The efficacy of AC was also found to be significantly lower than the observed values for imarsil® and it is in agreement with Denli and Okan (2006), who suggested that it was due to the difference in the cationic compounds of chemical content of the charcoal used. This result was not in consistence with the results of tests on feedstuffs, where a higher recoveries trend of 84.0% was observed using AC which can be attributed to a high affinity of the activated carbons *versus* the AFB1 molecule. Abundance of micropores in activated carbons increases its adsorption properties (Kumar and Jena 2016).

Although the estimated total aflatoxin in the oil samples adhered to standard regulatory limits, but there is no threshold at which no harmful effect occurs. Its accumulation in the liver poses a significant hazard to both human and animal health, giving rise to a multitude of chronic health hazards such as cancer induction, as well as defects in diges-

tion, blood, and nerves (Rizzi *et al*., 2003).

CONCLUSION

This present investigation has unveiled the potential of imarsil® as a proficient adsorbent in vegetable oil processing industries, offering a promising avenue for mitigating and ameliorating the prevalence of aflatoxicosis.

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Conflict of interest
The authors declare that there are no conflicts of interest.

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