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Comparative Efficacies of Imarsil and Activated Charcoal in Reducing Aflatoxin M_1 in Cows' Milk

Flora Oluwafemi [α], Aminat. Badmos [σ], Adelodun Kolapo [ρ], Sarafadeen Kareem [ω] & Oladipo Ademuyiwa[¥]

Abstract- **Many health risks associated with Aflatoxin M_1 (AFM_1) drive the demand for its control. Adsorption studies of AFM_1 were performed using activated charcoal (AC) and imarsil (0.5, 1 or 2%) at aflatoxin contamination rates of 9, 231 or 456 ng/L for 5 h at 4, 16, 28 and 32ºC. The aflatoxin-adsorbing capabilities of the two adsorbents depend on the adsorbent, adsorbent concentration, contact time and treatment temperature. At 4, 16 and 28ºC, Imarsil demonstrated significant reductions ($p<0.05$) at the highest contamination rate and adsorbent concentration; while at 32ºC, significant reductions ($p<0.05$) were observed at all contamination rates and adsorbent concentrations. However, at all treatment temperatures AC exhibited a very poor adsorbent capacity, except at 32ºC where a mild activity was only exhibited at the highest contamination rates and adsorbent concentration. Results from the present study indicate that imarsil demonstrates a potential for aflatoxin reduction in the developing tropical world.**

Keywords: cow's milk, aflatoxin M_1, HPLC, adsorbents, activated charcoal, imarsil.

I. Introduction

Milk is an important component of human diets. Milk plays an important role in nutrition, growth, development and immunity (Keira and Mao, 2004). The composition of milk varies from species to species (Kataoka, 2002). Although cow's milk has continued to play an important role in human nutrition, growth and development, raw milk obtained from cows may contain a wide range of contaminants. Galvano *et al.* (2001) reported that milk, regarding mycotoxin, is mainly contaminated with aflatoxin M_1 (AFM_1), and consumption of such milk might be a principal route for entrance of AFM_1 into the human body.

Aflatoxins are fungal metabolites that contaminate the food supply in certain areas of the world (Gourama and Bullerman, 1995; Smela *et al.,* 2001). The contamination of food with aflatoxins is more serious in tropical countries, where relative humidity is high, and the temperatures are conducive to the growth and production of aflatoxins by moulds. These toxins are produced by *Aspergillus flavus*, *A. parasiticus* and *A. normius*, which grow on improperly stored foods. Aflatoxin M_1 is a toxic metabolite of aflatoxin B_1 (AFB_1): it is formed by enzymatic hydroxylation of the B_1 carried over from contaminated feed, primarily cereal grains. It is normally excreted in the urine and also in the milk of dairy cattle (Creepy, 2002; Gurbay *et al.,* 2006; Oliveira and Ferraz, 2007). In a related observation, AFM_1 is present in the milk of nursing women who eat food containing AFB_1 (Henry *et al.,* 2001; Oluwafemi, 2012). The occurrence of AFM_1 in milk is transitory in nature, usually reaching a peak within two days after the ingestion of the contaminated commodity and disappears within 4-5 days after the withdrawal from a contaminated food source (Henry *et al.,* 2001).

The major concerns with aflatoxins are their potent carcinogenic, mutagenic and teratogenic effects in humans (Battacone *et al.*, 2003, Kocabas and Sekerel, 2003). Although AFM_1 is less carcinogenic and mutagenic than AFB_1, it exhibits a high level of genotoxic activity and certainly represents a health risk due to its possible accumulation and linkage to DNA (Shundo and Sabino, 2006). Avoidance of contaminated food/feed is rarely possible, and feeds that contain relatively low concentrations of AFB_1 may still have deleterious effects on sensitive species such as poultry (Doer et al., 1983; Giambrone *et al.,* 2005; Rauber *et al.*, 2007). Unfortunately, residues of AFB_1 and AFM_1 in different animal products, such as meat, eggs and milk, intended for human consumption have been reported (Pattersen *et al.,* 2005; Mumksgaard *et al.,* 2007; Oluwafemi, 2012).

Recently, several approaches to preventing aflatoxins from entering the food chain such as decontamination or remediation of feed and feedstuffs have been proposed (Bailey *et al.,* 1998; Ledoux *et al.*, 1999). A variety of adsorbents such as bentonite (Rosa *et al.*, 2001), zeolite (Miazzo Roso *et al.*, 2000), hydrated sodium calcium aluminosilicate (HSCAS) (Kubena *et al.,* 1993; Scheideler, 1993; Ramos and Hernandez, 1997), clinoptilolite (Oguz *et al.*, 1994), dietary clay (Philips, 1999), *Saccharomyces cerevisiae* (Celik *et al.*, 2001) and activated charcoal (AC) (Jindal *et al.,* 1994) have been used successfully to detoxify AFB_1 in contaminated feeds. In a very recent study, Manafi and Khosravinia (2013) reported on the efficacy of an herbal mycotoxin binder (a unique combination of minerals,

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antioxidants and enzymes) for the control of aflatoxin in broiler/breeder diets.

Little attention has been paid to studies on adsorption of AFM_1 from milk meant for human consumption. This study is reasonable given the understanding that the control of aflatoxin in feed will automatically translate into alleviation of AFM_1 in dairy cattle that are fed such remediated feed (Smith et al., 1994). However, it is pertinent to note that this wide assumption is hardly applicable to free-range dairy cattle because the diet of such cattle is difficult to monitor or control. Incidentally, a large portion of dairy cattle in many developing countries such as Nigeria are raised under a free-range system. As such, control measures for aflatoxin in products obtained from those animals should be designed, and this is the goal of the present study.

Imarsil™, is an inexpensive synthetic absorbent obtained from oxidized natural polymer of *Brachystegia nigerica* (Akpan and Kareem, 2002) *B. nigerica* is a legume used especially in the eastern states of Nigeria as condiment to thicken soup. Its thickening characteristics have been attributed to the presence of hydrocolloid property or gelling property (Odum, 2000). Imarsil as an oxidized polymer of *B. nigerica* is considered as an efficient absorbent because of its quick and simple recovery approach especially in the clarification of microbial enzymes from fermentation broth (Kareem and Akpan, 2003).

The objective of the present study is to compare the efficacy of easily and locally accessible adsorbents, such as imarsil and activated charcoal, in the reduction of AFM_1 contamination of milk.

II. Materials and Methods

a) Chemicals and standards

Imarsil™ was prepared as previously described by Akpan and Kareem (2002). Solvents such as acetonitrile, methylene chloride, and methanol were of HPLC grade. The standard AFM_1 was purchased from Chromogen (New Delhi, Delhi, India). Immunoaffinity column was Aflastar M1 R, (lot: AF 1011 1012), supplied by Roma Labs Diagnostic Technopark 13430, Tulln, Austria.

b) Adsorption Studies

The adsorption studies of aflatoxin were performed using a 4 x 4 x 2 x 3 x 3 factorial design involving four different contact times (2, 3, 4, or 5 h), four temperature levels (4, 16, 28, or 32°C), two adsorbents (activated charcoal or imarsil) at three different concentration (0.5, 1 or 2%) and three concentrations of aflatoxins (9, 231 or 456 ng/L AFM_1).

Recent report of survey of AFM_1 in cows' milk from free-grazing cows in Nigeria indicated that toxin levels in positive samples ranged from 9.0 to 456.0 ng/L (Oluwafemi *et al.*, and 2014). Therefore, milk samples (50mL) were spiked with AFM_1 at the three concentrations of 9, 231 and 456 ng/L AFM_1. These were passed through a separating funnel containing activated charcoal and imarsil at three different concentrations (0.5, 1 or 2 %). The experimental setups were in place for 5 hrs with samples taken at 2, 3, 4 and 5 hrs. The experiment was repeated at four different temperatures: 4, 16, 28 and 32°C. Quantification of AFM_1 in the remediated milk samples was performed by a modification of the method of Smith *et al* (1994) as recently described by Oluwafemi *et al.* (2014).

c) Nutrient analysis

The nutrient contents such as moisture, protein, fat, carbohydrates and saturated fatty acid were determined using the AOAC method (1993) before and after the administration of the adsorbents.

d) Statistical analysis

The comparisons between means were evaluated using Student's t-test and analysis of variance. A value of $p < 0.05$ was considered significant.

III. Result and Discussion

The efficacy of the different tested adsorbents in reducing the AFM_1 in the contaminated milk samples is shown in Figures1a -4 b. The aflatoxin-adsorbing capabilities of the two investigated adsorbents depend on the adsorbent, adsorbent concentration, contact time and treatment temperature. At the investigated temperatures of 4, 16 and 28°C, Imarsil demonstrated a significant reductions ($p<0.05$) of AFM_1 contents of contaminated milk at the highest contamination rate (0.456 µg/l) and adsorbant concentration(2.0%) (Figures 1a, 2a and 3a). However, at 32°C significant reductions ($p<0.05$) were observed at all contamination rates and adsorbant concentrations (Figure 4a). On the contrary, at all treatment temperatures AC exhibited a very poor adsorbant capacity, except at 32°C where a mild activity was exhibited only at the highest contamination rates and adsorbant concentration (Figures 1b, 2b, 3b and 4b).

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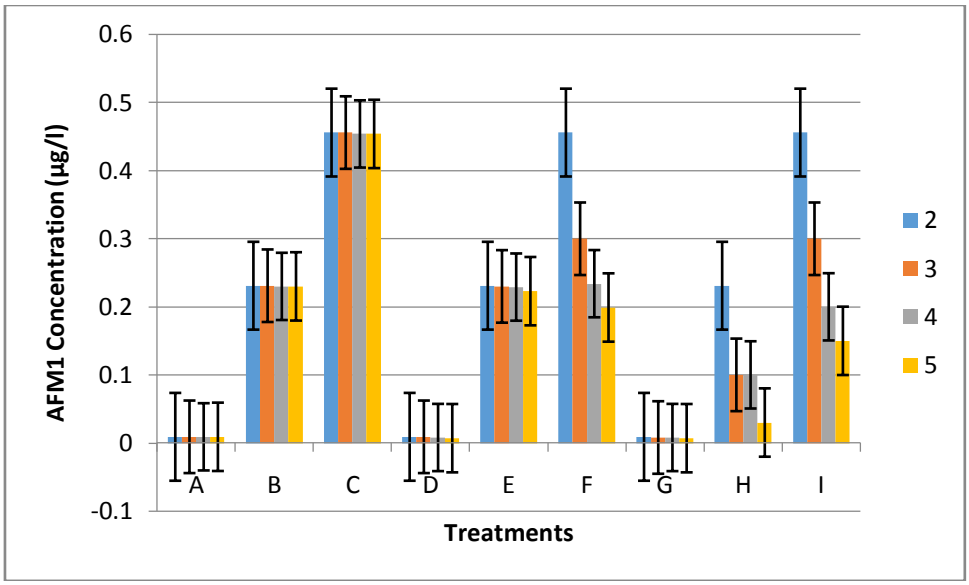

Figure 1a : Effect of Imarsil on AFM1 content of Contaminated milk treated for 5 hrs at 4C

KEY

A=0.009 µg/lAFM_1 +0.5 % Imarsil; B=0.231 µg/l AFM_1 +0.5 %Imarsil; C= 0.456 µg/l AFM_1 +0.5 %Imarsil
D=0.009 µg/l AFM_1 +1.0 % Imarsil; E=0.231 µg/l AFM_1 +1.0 % Imarsil; F=0.456 µg/l AFM_1 +1.0 % Imarsil;
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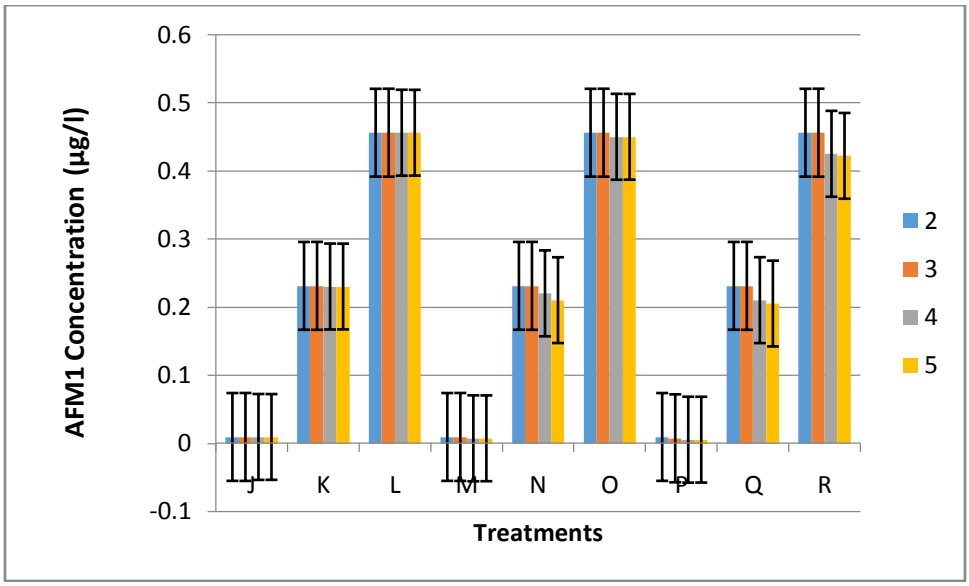

Figure 1b : Effect of Charcoal on AFM1 content of Contaminated milk treated for 5 hrs at 4C

KEY

J=0.009 µg/lAFM_1 +0.5 % Charcoal; K=0.231 µg/l AFM_1 +0.5 % Charcoal; L= 0.456 µg/l AFM_1 +0.5 % Charcoal
M=0.009 µg/l AFM_1 +1.0 % Charcoal; N=0.231 µg/l AFM_1 +1.0 % Charcoal; O=0.456 µg/l AFM_1 +1.0 % Charcoal;
P=0.009 µg/l AFM_1 +2.0 % Charcoal; Q=0.231 µg/l AFM_1 +2.0 % Charcoal; R=0.456 µg/l AFM_1 +2.0 % Charcoal

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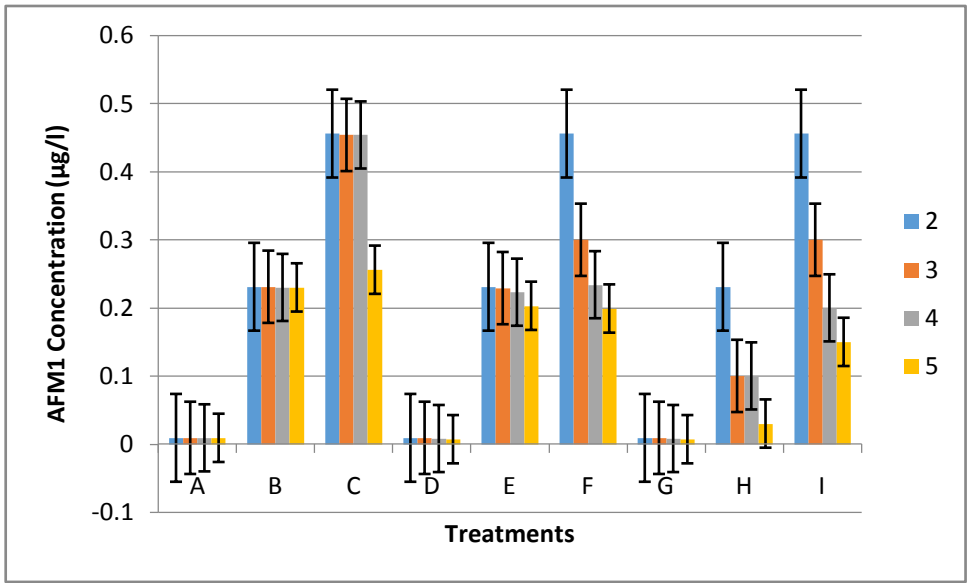

Figure 2a : Effect of Imarsil on AFM1 content of Contaminated milk treated for 5 hrs at 16C

KEY

A=0.009 µg/lAFM$_1$ +0.5 % Imarsil; B=0.231 µg/l AFM$_1$ +0.5 %Imarsil; C= 0.456 µg/l AFM$_1$ +0.5 %Imarsil
D=0.009 µg/l AFM$_1$ +1.0 % Imarsil; E=0.231 µg/l AFM$_1$ +1.0 % Imarsil; F=0.456 µg/l AFM$_1$ +1.0 % Imarsil;
G=0.009 µg/l AFM$_1$ +2.0 % Imarsil; H=0.231 µg/l AFM$_1$ +2.0 %Imarsil; I=0.456 µg/l AFM$_1$ +2.0 % Imarsil

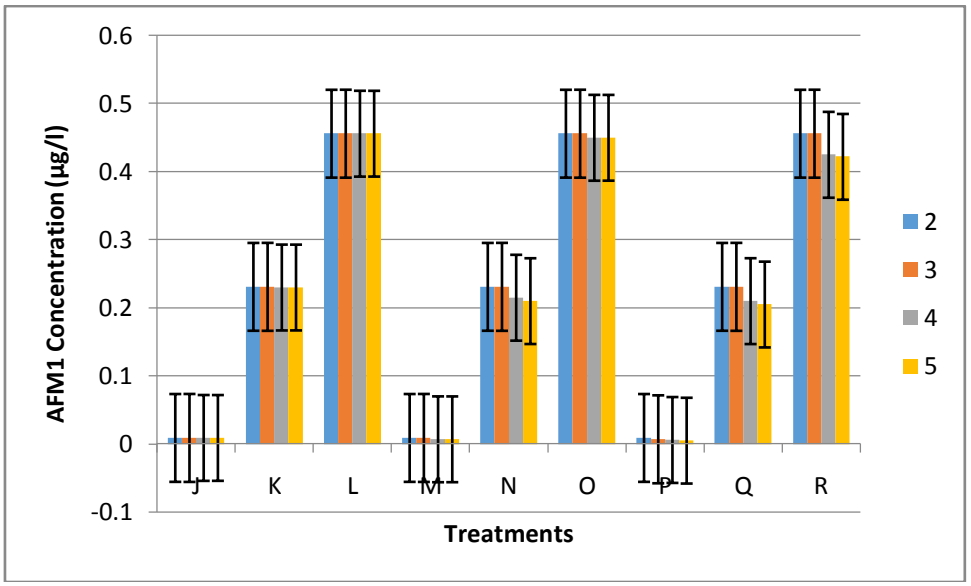

Figure 2b : Effect of Charcoal on AFM1 content of Contaminated milk treated for 5 hrs at 16C

KEY

J=0.009 µg/lAFM$_1$ +0.5 % Charcoal; K=0.231 µg/l AFM$_1$ +0.5 % Charcoal; L= 0.456 µg/l AFM$_1$ +0.5 % Charcoal
M=0.009 µg/l AFM$_1$ +1.0 % Charcoal; N=0.231 µg/l AFM$_1$ +1.0 % Charcoal; O=0.456 µg/l AFM$_1$ +1.0 % Charcoal;
P=0.009 µg/l AFM$_1$ +2.0 % Charcoal; Q=0.231 µg/l AFM$_1$ +2.0 % Charcoal; R=0.456 µg/l AFM$_1$ +2.0 % Charcoal

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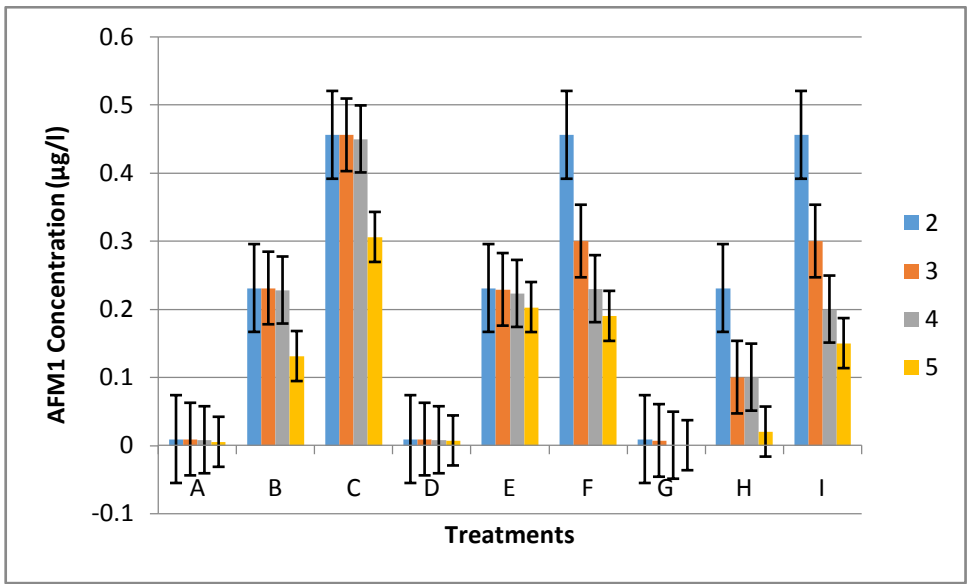

Figure 3a : Effect of Imarsil on AFM1 content of Contaminated milk treated for 5 hrs at 28C

KEY

A=0.009 µg/lAFM$_1$ +0.5 % Imarsil; B=0.231 µg/l AFM$_1$ +0.5 %Imarsil; C= 0.456 µg/l AFM$_1$ +0.5 %Imarsil
D=0.009 µg/l AFM$_1$ +1.0 % Imarsil; E=0.231 µg/l AFM$_1$ +1.0 % Imarsil; F=0.456 µg/l AFM$_1$ +1.0 % Imarsil;
G=0.009 µg/l AFM$_1$ +2.0 % Imarsil; H=0.231 µg/l AFM$_1$ +2.0 %Imarsil; I=0.456 µg/l AFM$_1$ +2.0 % Imarsil

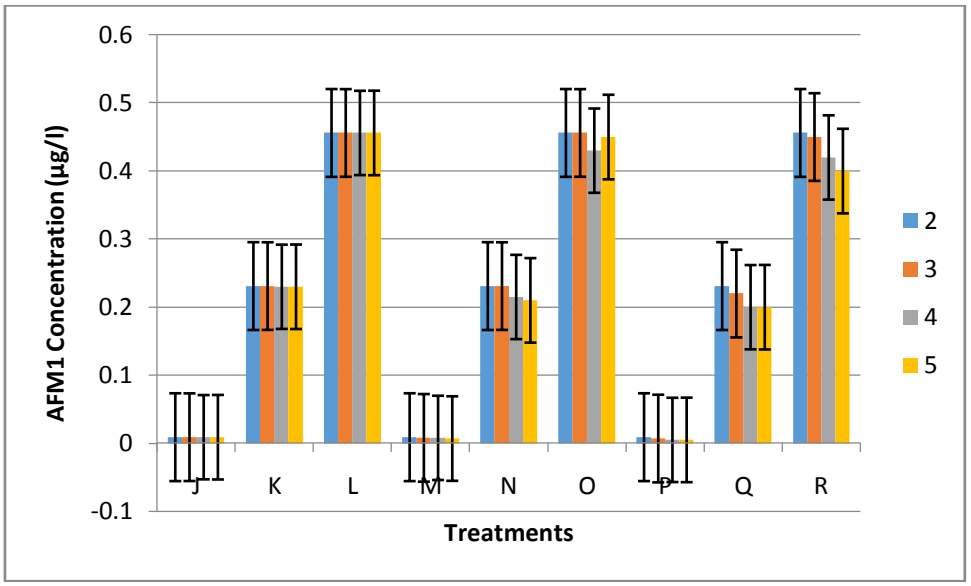

Figure 3b : Effect of Charcoal on AFM1 content of Contaminated milk treated for 5 hrs at 28C

KEY

J=0.009 µg/lAFM$_1$ +0.5 % Charcoal; K=0.231 µg/l AFM$_1$ +0.5 % Charcoal; L= 0.456 µg/l AFM$_1$ +0.5 % Charcoal
M=0.009 µg/l AFM$_1$ +1.0 % Charcoal; N=0.231 µg/l AFM$_1$ +1.0 % Charcoal; O=0.456 µg/l AFM$_1$ +1.0 % Charcoal;
P=0.009 µg/l AFM$_1$ +2.0 % Charcoal; Q=0.231 µg/l AFM$_1$ +2.0 % Charcoal; R=0.456 µg/l AFM$_1$ +2.0 % Charcoal

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Imarsil exhibited 0.4- 87% reduction at 4 and 16°C, while 12-100 and 22-100% reduction in AFM_1 were observed at 28 and 32°C, respectively. The efficacy of AC was significantly ($p<0.05$) lower than the observed values for imarsil: this adsorbent only achieved reductions of 0.4-22% at 4 and 28°C and 0.4-44 and 0.4-77% at 16 and 32°C, respectively. AFM_1 removal is thus concentration-, time- and temperature-dependent.

Previous studies that evaluated the ability of AC to reduce AFB1 toxicity have produced conflicting results. Studies by Dalvi and Ademoyero (1984), Dalvi and McGowan (1984) and Hesham *et al.* (2004) indicated that AC was able to decrease aflatoxin toxicity, while Kubena *et al.* (1990), Edrington *et al.,* (1996) and Denli and Okan (2006) reported no significant differences in performance of birds following the addition of charcoal to rations that included aflatoxins. Denli and Okan (2006) had suggested that the conflicting results could have been due to the differences in chemical content, specifically the cationic compounds of the charcoal used. In the present study, it is evident that AC demonstrated a comparatively weak efficacy for AFM_1 removal. Hence, it is also probable that the previously observed conflicting results regarding the efficacy of AC to adsorb aflatoxin could be as a result of failure to standardize the experimental conditions such as concentrations of aflatoxin, adsorbent and detection methods.

The moisture, protein, fat, carbohydrates and saturated fatty acid contents of the investigated milk before the adsorption studies were 87.8, 3.2, 3.9, 4.8 and 1.4% respectively. After remediation with both Imarsil and AC, the nutrient contents of remediated milk were the same, suggesting that these adsorbents had no effect on the essential nutrients of milk.

Earlier reports have demonstrated that imarsil possesses a flocculating ability, and hence it has been used for enzyme purification (Kareem and Akpan, 2003; Kareem and Adebowale, 2007). Economic and logistical considerations sometimes restrict the use of conventional techniques to detoxify aflatoxin (Smith *et al.* 1994). The use of high affinity adsorbents such as imarsil to significantly diminish the AFM_1 content of contaminated milk as shown in the present study therefore represents a notable approach to the management of aflatoxicosis in the developing tropical world.

IV. Conclusions

The results from the present study indicate that imarsil demonstrates a potential to reduce aflatoxin M_1 and by extension ameliorate the risk of aflatoxicosis in the developing tropical world. However, further studies to evaluate imarsil's in vivo activity, its effect on biochemical indicators in both humans and animals and the organoleptic qualities of the remediated milk will be needed.

V. Acknowledgments

F. Oluwafemi acknowledges the Federal University of Agriculture, Abeokuta through IFSERAR for research grant No. RG101 and the Mycotoxin Laboratory of the National Agency for Food, Drug, Administration and Control (NAFDAC) for providing state-of-the-art equipment for aflatoxin M_1 quantification.

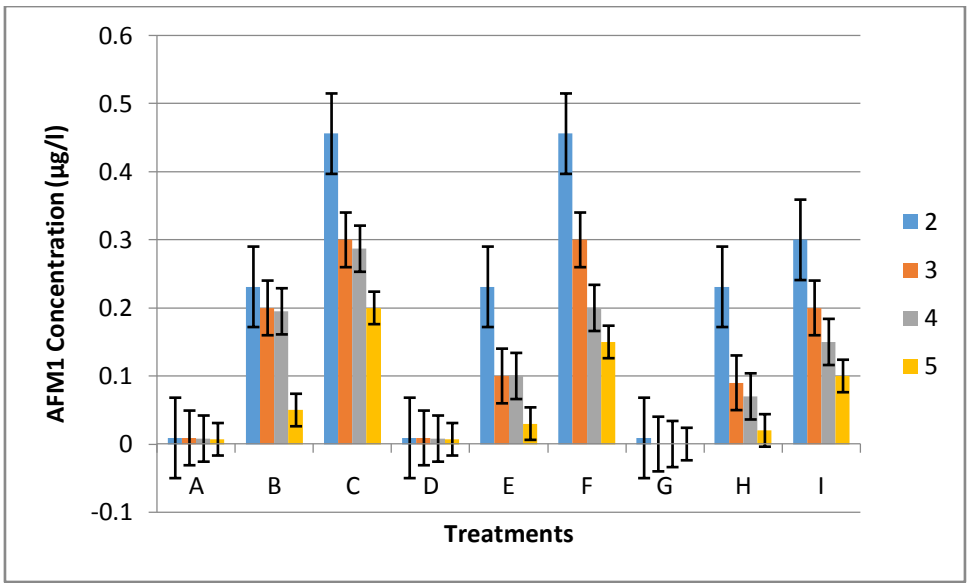

Figure 4a : Effect of Imarsil on AFM_1 content of Contaminated milk treated for 5 hrs at 32C

KEY

A=0.009 µg/lAFM_1 +0.5 % Imarsil; B=0.231 µg/l AFM_1 +0.5 %Imarsil; C= 0.456 µg/l AFM_1 +0.5 %Imarsil

D=0.009 µg/l AFM_1 +1.0 % Imarsil; E=0.231 µg/l AFM_1 +1.0 % Imarsil; F=0.456 µg/l AFM_1 +1.0 % Imarsil;

G=0.009 µg/l AFM_1 +2.0 % Imarsil; H=0.231 µg/l AFM_1 +2.0 %Imarsil; I=0.456 µg/l AFM_1 +2.0 % Imarsil

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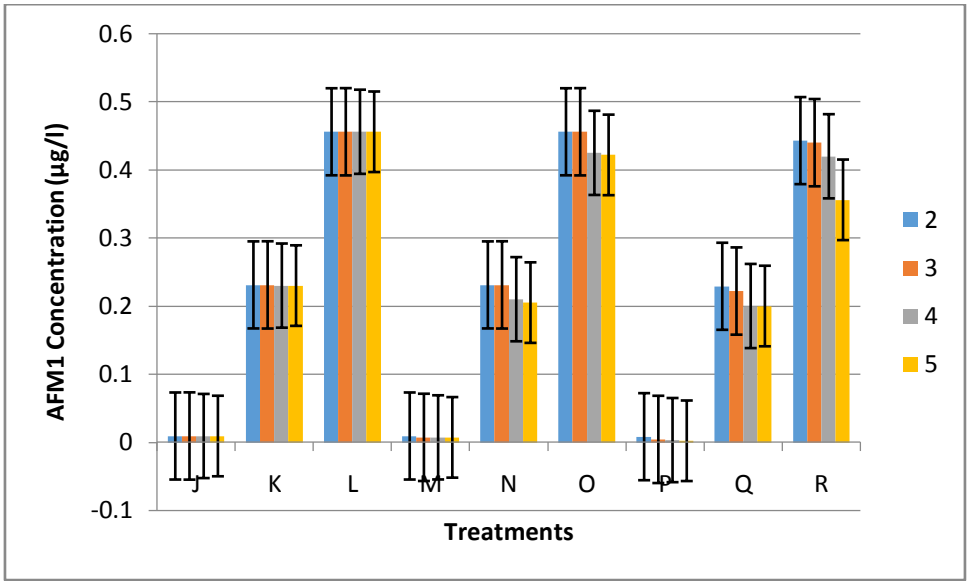

Figure 4b : Effect of Charcoal on AFM1 content of Contaminated milk treated for 5 hrs at 32C

KEY: As in Fig 3b

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