



SJMLS-2(3)-2017-022

Aflatoxin (M_1 and B_1) Contamination of Locally Repacked Milk Powder in South-Western Nigeria.

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Abstract

Aflatoxins are toxic fungal metabolites found in foods and feeds. When mammals eat aflatoxin B_1 (AFB_1)-contaminated foods/feeds, they metabolize the toxin and excrete aflatoxin M_1 (AFM_1) in milk. This paper reports the levels of aflatoxins M_1 (AFM_1) and B_1 (AFB_1) contamination in one hundred and twenty-five locally repacked powdered milk samples from five States in the South-Western region of Nigeria. Repacked milk samples in polyethylene bags (nylons) and plastic containers were purchased and assessed for fungal contaminants. Samples were analyzed for AFM_1 using a rapid diagnostic kit (Aflasensor), Immunoaffinity Column (IAC) and High-Performance Liquid Chromatography (HPLC) with fluorescence detection, for comparison. AFB_1 was screened using IAC/HPLC. *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus* and *Mucor* species were the predominant fungal isolates. AFM_1 was detected in 41 (32.8%) and 67 (53.6%) samples by the Aflasensor kit and IAC/HPLC, respectively, at levels ranging from 0.02 - 0.46 $\mu\text{g}/\text{kg}$. AFB_1 was found in 119 (95.2%) samples analyzed at concentrations of 29.7-79.4 $\mu\text{g}/\text{kg}$. Whereas the AFM_1 found in the repacked milk powder samples were below the standard 0.5 $\mu\text{g}/\text{kg}$ permitted by European Union, Codex Alimentarius Commission and National Agency for Food and Drug Administration and Control (NAFDAC), in Nigeria, the AFB_1 levels were generally above the permissible limits of these regulatory agencies (4 - 5 $\mu\text{g}/\text{kg}$) for most foods.

Key words: Fungi, aflatoxin (M_1 and B_1), aflasensor kit, repacked milk powder, safety, NAFDAC

Introduction

Humans are continuously exposed to different amounts of chemicals that have been reported to have carcinogenic, hepatotoxic, nephrotoxic, or mutagenic properties in environmental systems. Exposure can occur exogenously when these agents are present in food, air or water, and also endogenously when they are products of metabolism or pathophysiologic states such as inflammation. Great attention is focused on environmental health in the past two decades as a consequence of the increasing awareness over the quality of life due to major environment pollutants that affect it. Studies have shown that exposure to environmental chemical carcinogens have contributed significantly to cause human cancers, when exposures are related to life style factors such as diet (IARC, 2012; Oyeyipo *et al.*, 2012).

Aflatoxins are potent toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites by fungus of the genus *Aspergillus* on variety of foods and food products. The main aflatoxin-producing species are *Aspergillus flavus* and *Aspergillus parasiticus*, although other species that produce aflatoxins in food have also been identified such

as *A. nomius* (Ozay *et al.*, 2008; Krutzman *et al.*, 1987), *A. tamarii* (Goto *et al.*, 1997), *A. pseudotamarii* (Ito *et al.*, 2001) and *A. australis* (IARC, 2002). Aflatoxins are fluorescent compounds with a condensed coumarinic structure with one bifurane and one pentanone (aflatoxins B) or one lactone (aflatoxins G) group. The letters B and G refer to the colour of the aflatoxins under ultraviolet light (Blue and Green), and the numbers 1 and 2 refer to their position in chromatographic separation. The more relevant aflatoxins in terms of food safety are B1 and B2 (produced by *A. flavus* and *A. parasiticus*), G1 and G2 (produced by *A. parasiticus*) and M1 and M2 (metabolites of aflatoxins B1 and B2, respectively, which are excreted in milk). Among them, aflatoxin B1 stands out from a health safety point of view as it is the most common in food as well as the

most toxic for humans (Deng *et al.*, 2010; Reddy *et al.*, 2008; Martins *et al.*, 2007). AFB₁ is the most known potent natural carcinogen. The activation of AFB₁ by phase I enzymes namely, cytochrome P450 (CYPs) isoenzymes, CYP1A2 and CYP3A4, produces AF1-8, 9-epoxide which is highly carcinogenic in humans. It forms DNA adducts and albumin adducts (ICMSF, 1996; Lamplugh, 1980). A reactive glutathione *S*-transferase (GST) system found in the cytosol and microsomes catalyzes the conjugation of activated aflatoxins with reduced glutathione, leading to the excretion of aflatoxin. Variations in the level of the GST system as well as variations in the CYP system are thought to contribute to the differences observed in interspecies aflatoxin susceptibility (IARC, 2012; Oyeyipo, 2012).

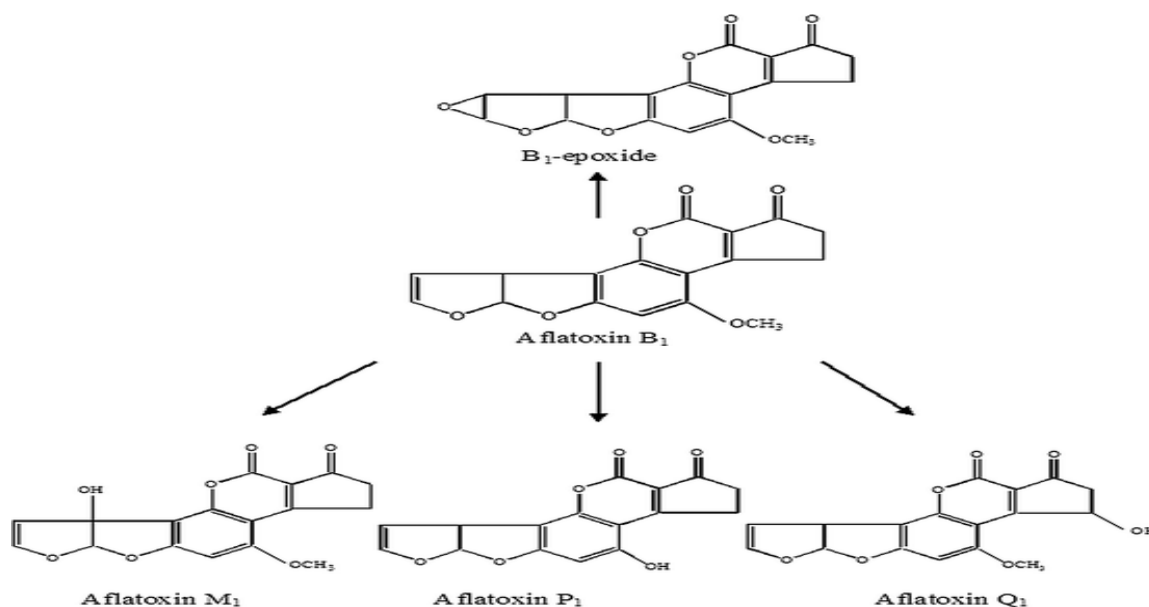


Figure 1: Metabolic pathways of AFB₁ by cytochrom P-450 enzymes; B1 epoxide = AFB₁ epoxide, M1 = aflatoxin M1, P1 = aflatoxin P1 and Q1 = aflatoxin Q1.

Until recently, only milk powder in their manufacturer’s original packaging materials (tins and aluminium-coated bags) was sold in the open markets. However, it is now common practice for some local retailers to devise means of repackaging them in different materials for sale in open markets, perhaps, due to the difficult economic situations in many

developing countries and especially in Nigeria. This repacked milk powder is utilised in various ways: fortifying weaning foods for growing children, baking, and in some instances direct consumption by children and adults (which may include expectant mothers). Human and other animal exposure to levels of aflatoxins from nanograms to micrograms per day occurs



through consumption of foods contaminated with AFB₁ and can excrete a hydroxylated metabolite of AFB₁ as AFM₁ in milk (Chopra *et al.*, 1999; Frobish *et al.*, 1986). AFM₁ has been classified as a second-class possible human carcinogen by the IARC (2012). Moreover, its carcinogenicity was estimated to be 1/10th of that of AFB₁ by the Joint Expert Committee on Contamination and Food Additives in 2001 (Sugiyama *et al.*, 2008). Furthermore, AFM₁ is very stable, and it is assumed that neither storage nor processing, such as pasteurisation, autoclaving, fermentation or other methods commonly used to process milk products, destroys its toxin (CEC, 2010; Tajkarimi *et al.*, 2008; Park, 2002; ICMSF, 1996).

The human gastrointestinal tract rapidly absorbs aflatoxin after consumption of contaminated foods and the circulatory system transports aflatoxin to the liver (Fung and Clark, 2004). From 1-3% of ingested aflatoxin irreversibly bind to proteins and DNA bases to form adduct such as aflatoxin B₁ lysine in albumin. Disruption of protein and DNA bases in hepatocytes causes liver toxicity (Tandon *et al.*, 1978). Early symptoms of hepatotoxicity from aflatoxicosis can manifest as anorexia, malaise, and low-grade fever. Aflatoxicosis can progress to potentially lethal acute hepatitis with vomiting, abdominal pain, hepatitis and death (Etzel, 2002). Symptoms of B₁ also include yellow eyes, swollen legs, vomiting, abdominal pain and bleeding. The health impact of aflatoxin exposure in animals mainly depends on dosage and response. Low dosages produce nutritional interference and immunological suppression, while high doses lead to acute illness and death (Lunyasunya *et al.*, 2005). Aflatoxins have been detected in the blood of pregnant women, in neonatal umbilical cord blood, and in breast milk in African countries, with significant seasonal variations (Oluwafemi, 2012; Maxwell *et al.*, 1989). Levels of aflatoxins detected in some umbilical cord blood at birth are among the highest levels ever recorded in human tissues and fluids (Coulter, 1984). In one study, aflatoxin was found in the brains and lungs of children who have died from kwashiorkor and those who had died from

various other diseases (Oyelami., 1995). Approximately 95% of AFB₁ metabolites excreted in milk are in the form of AFM₁, though AFM₂, AFG₁ and AFB₁ have been reported (IARC, 2012; Oluwafemi, 2012).

There is little or no data on the fungal and mycotoxin contamination of locally repacked milk powder under market conditions in Nigeria. Data available were generally on the microbial quality and AFM₁ levels of milk powder (infant formula and whole milk/cream powder) in their original manufacturer's packaging materials (tins or aluminium coated bags). Therefore, this paper reports the fungal-quality, Aflatoxin (M₁ and B₁) levels and public health risks associated with locally repacked milk powder under market conditions in South-Western Nigeria.

Materials and Methods

Source of samples

A total of one hundred and twenty-five (125) samples of milk powder locally repackaged in polyethylene bags (nylons) and transparent plastic containers (of various sizes), were purchased randomly from different locations in the open markets in five States (Lagos, Ogun, Osun, Oyo and Ekiti) of South-Western Nigeria. Twenty-five (25) samples from each State were procured on two different occasions. Samples were aseptically collected into a 150ml-capacity, wide-mouthed glass containers with air-tight fitting caps, which were previously disinfected with ethyl alcohol and autoclaved at 121°C for 15 minutes. The samples were immediately transported to the laboratory for analyses.

Preparation of samples and isolation of fungi

The protocol of Houghty *et al.* (1992) and Christen *et al.* (1992) were adopted. Twenty-five grams (25g) of the repacked milk powder sample was aseptically (cleaning the tied mouth/cover of the polyethylene bags or plastic containers, respectively with ethyl alcohol) weighed and introduced into 225 ml of previously-sterilized buffered peptone water (LAB M). Control sample (milk powder in its manufacturer's original packaging material) was

also subjected to similar treatment. The mixture (solution) was homogenized by shaking vigorously to produce the homogenate. Subsequently, serial 10-fold dilutions of samples were prepared and 0.1ml aliquots were spread plated on acidified (pH 3.5) potato dextrose agar (PDA, Unipath, Basingstoke Ltd, UK). The plates (in duplicates) were incubated at 28°C for 5 - 7 days. Each of the fungi that emerged was sub-cultured onto a fresh PDA slant in a 15-ml slant bottle to obtain a pure culture. Each pure culture was characterized and identified on the basis of their morphological and microscopic characteristics using the protocols of Samson and van Reenen-Hoekstra (1988).

Direct detection of Aflatoxin M₁ in repacked milk powder using Aflasensor kit

Composition and use of the Aflasensor kit

Aflasensor (Unisensor, Belgium) is a rapid diagnostic/semi-quantitative test that allows the detection of the presence of aflatoxin M₁ (AFM₁) molecules in milk samples. This dipstick assay does not require sample processing, cleaning or extraction. The test takes about 10 minutes at 40°C (using heat sensor). The result is directly interpreted by visual observation with threshold level at 100 parts per million (ppm) of AFM₁. The limit of quantification (LOQ) of the Aflasensor is 20 ppt (part per trillion) with a quantification range from 20 to 150 ppt. Aflasensor diagnostic kit contain materials for performing 96 measurements/tests. 12 pots, 8 reagents micro-well, 8 dipsticks, one micropipette of 200µl, positive and negative standards and a heat sensor for incubating samples. The Aflasensor is a competitive test involving specific antibody with high affinity for aflatoxin M₁ molecules. The test requires the use of two components: a micro-well containing predetermined amount of antibody linked to gold particles, and a dipstick made up of a set of membranes with specific capture lines.

Procedure for Aflasensor

Two hundred (200) µl of aseptically reconstituted repacked milk powder sample (homogenized milk powder samples in sterile

deionised water) was added into the reagent in micro-well and agitated vigorously for homogeneity. The sample was incubated for 3 minutes at 40°C in the heat sensor. The negative and positive control standards were treated alike. The dipstick was dipped into the reagent micro-well. The second incubation continued for 7 minutes at 40°C. The colour intensities of the test line on the dipstick was read and compared with the control line.

Interpretation of result

When the colour of the test line was lighter, same intensity or darker than the control line, the result was: faintly positive (+), positive (++) and full positive (+++), respectively. Then sample was considered to contain aflatoxin M₁ at lower, equal or higher concentration than 100ppt. When no test line was observed, the sample contained no Aflatoxin M₁ residues and considered as negative (-) (< 20ppt) (Figure 5).

Extraction of aflatoxin from locally repacked milk powder samples

Extraction was done as previously described (Oluwafemi, 2012; Lamplugh 1980) with little modification. Briefly, milk powder was reconstituted and homogenized with sterile distilled water (1:5). 1-ml of hexane was added to each sample and centrifuged (3000 x g, 5min) and the hexane layer removed. This was repeated twice for each sample. Thereafter, 1-ml of chloroform was added to the defatted milk and centrifuged (4,000 x g, 10min). The chloroform layer was collected into plastic bottles. This was repeated four times and chloroform layer pooled together. The extracts were kept in a dark cup-board overnight to dry.

Analysis of AFM₁ and AFB₁

Reagents used were of HPLC grade or equivalent. All water used was distilled and, for HPLC, passed through a Milli-Q purification system (Millipore, London, UK). Acetonitrile used for mobile phase was of HPLC grade and provided by Merck, Darmstadt, Germany.

Immunoaffinity columns (IACs) (RIDA Aflatoxin column, R-BIOPHARM, Darmstadt, Germany) were used for cleaning the sample



extracts. The IAC was brought to room temperature, plugged unto Luer-attachment of a vacuum pressure facility. Twenty-five grams of each powder milk sample was weighed into a 250-mL Erlenmeyer flask, 125 mL of acetone/water (85:15, v/v) was added and placed on a magnetic stirrer (Telemodule, Labortechnik) at 500 rpm for 45 minutes, and 5 mL of the filtrate was measured into a vessel connected to the IAC. Forty-five mL of Millipore water was added to the barrel attached to the IAC. The sample was allowed to flow through the IAC at a rate of ca. 1 mL per minute. Slight pressure was applied. The reservoir was rinsed twice with 10 mL of phosphate buffer at a flow rate of ca. 2-3 mL per minute. Then the reservoir was removed, and the IAC was dried by applying pressure. AFM₁ was collected in glass bottles previously treated with 2N H₂SO₄. The solvent used for eluting was 1.25 mL methanol/acetonitrile (20:30, v/v). The eluate was dried in a water bath at 40 °C and 80 kPa. The dried extract was re-dissolved with 1.25 mL of acetonitrile: water (25:75, v/v). The acetonitrile/water solution was used as the mobile phase for HPLC. AFB₁ were also determined. The HPLC system consisted of a LDC, Milton Roy, Consta Metric 1 pump and a Lichrosorb RP-18 (Merck Hibar, UK) column (particle size of 5 µm, length, 125 mm, inside diameter, 4mm). The pump pressure was 60MPa. The Injector was automatic type (Rheotype, Gilson Abimed Model 231). The detector had a fluorescence spectrometer (Shimadzu RF 535, gamma excitation, 365 nm, gamma emission, 44nm). The flow rate was 1ml min⁻¹ and the injection volume was 50µl. The mobile phase was water/acetonitrile (75:25).

Standard solutions of AFM₁ and AFB₁

Aflatoxins M₁ and B₁ was obtained from Sigma-Aldrich (USA). The commercial stock solution of AFM₁ and AFB₁ was 1000ng/ml. stock solution 1:40 approximately 25ng/ml using HPLC grade acetonitrile/water. Out of the diluted stock solution, 140 µl were added to 70ml of defatted repacked powdered milk. Calibration curve was prepared by diluting 2µg/l of AFM₁ and AFB₁ in a 1:500 dilution. The concentrations of standards were prepared to give the following 1.5µg/kg, 1.0µg/kg and 0.25µg/kg. The stock solutions were stored at 4⁰ C when not in use.

Statistical analysis

Comparison between means was evaluated by student t-test and analysis of variance (ANOVA). A value of $p \leq 0.05$ was considered significant.

Results

Fungi isolated from locally repacked milk powder

Table 1 depicts the types and incidence of fungal isolates from repacked milk powder displayed for sale under market conditions in five States (Lagos, Ogun, Osun, Oyo and Ekiti), South-Western, Nigeria. The incidence data are presented as fungal isolates found per total number of samples screened. Three hundred and sixty-eight (368) fungal isolates belonging to five genera (*Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus* and *Mucor*) were identified in the repacked milk powder. *Aspergillus flavus* recorded the highest incidence (103/125) while *Aspergillus versicolor* had the lowest incidence (3/125).

Table 1: Incidence of fungi in locally repacked powder milk samples from five South-western States in Nigeria

Species	Incidence per State					Total sample infected
	Lagos	Ogun	Osun	Oyo	Ekiti	
<i>Aspergillus flavus</i>	19/25*	18/25*	24/25*	20/25*	22/25*	103/125*
<i>Aspergillus fumigates</i>	5/25	2/25	-	1/25	2/25	10/125
<i>Aspergillus terreus</i>	5/25	4/25	1/25	7/25	-	17/125
<i>Aspergillus parasiticus</i>	11/25	14/25	11/25	8/25	9/25	53/125
<i>Aspergillus niger</i>	3/25	11/25	5/25	8/25	10/25	37/125
<i>Aspergillus glaucus</i>	-	-	1/25	-	3/25	4/125
<i>Aspergillus versicolor</i>	-	2/25	1/25	-	-	3/125
<i>Fusarium spp</i>	13/25	17/25	21/25	11/25	14/25	76/125
<i>Penicillium spp</i>	6/25	7/25	4/25	5/25	9/25	31/125
<i>Rhizopus spp</i>	7/25	3/25	4/25	3/25	-	17/125
<i>Mucor spp</i>	4/25	1/25	2/25	6/25	4/25	17/125
Total	73/25	79/25	74/25	69/25	73/25	368/125

Key: Values are presented as fungal isolates per total number of samples analyzed, * = Number of samples collected, - = Not detected.

Comparison of AFM₁ results obtained by Aflasensor and IAC/HPLC

Table 2 shows the comparative AFM₁ results obtained by the rapid diagnostic Aflasensor kit

and IAC/HPLC. While the Aflasensor kit detected AFM₁ in 41(32.8%) samples the IAC/HPLC detected AFM₁ in 67 (53.6%) samples out of 125 screened samples.

Table 2: Comparative detection of AFM₁ in repacked milk powder samples by Aflasensor kit and IAC/HPLC

Technique	AFM ₁ per State					Total sample contaminated (%)
	Lagos	Ogun	Osun	Oyo	Ekiti	
Aflasensor	12/25*	10/25*	7/25*	8/25*	4/25*	41/125*(32.8%)
IAC/HPLC	19/25*	15/25*	14/25*	10/25*	9/25*	67/125* (53.6%)

Key: Values are presented as AFM₁ detected per total number of samples analyzed, AFM₁= Aflatoxin M₁, * = Number of samples screened, IAC = Immunoaffinity column, HPLC= High performance liquid chromatography.

AFM₁ levels in locally repacked milk powder samples

The level of AFM₁ in repacked milk powder

samples is presented in Table 3. Varied levels of AFM₁ were observed. Generally, the AFM₁ ranged from 0.02-0.46.



Table 3: AFM₁ concentrations in repacked milk powder samples from five States in South-Western Nigeria

State	Number of Sample	Number With AFM ₁	AFM ₁ range (µg/kg)	No. exceeding NAFDAC/EC/Codex (0.5µg/kg)
Lagos	25	19	0.02-0.46	None
Ogun	25	15	0.04-0.41	None
Osun	25	14	0.02-0.44	None
Oyo	25	10	0.03-0.39	None
Ekiti	25	9	0.02-0.40	None

Key: AFM₁= Aflatoxin M₁, NAFDAC= National Agency for Food and Drug Administration and Control, EC= European commission, Codex= Codex alimentarius commission.

AFB₁ level in locally repacked milk powder samples

The level of AFB₁ in repacked milk powder samples is shown in Table 4. High levels above

the standard approved limits occurred in the samples. AFB₁ level also varied from one sample/State to another. On the whole, the AFB₁ range was 29.7-79.4.

Table 4: AFB₁ concentrations in locally repacked milk powder samples from five States in South-Western Nigeria

State	Number Of Sample	Number with AFB ₁	AFB ₁ range (µg/kg)	No. exceeding NAFDAC/EC/Codex (5µg/kg)
Lagos	25	25	33.3-79.4	All
Ogun	25	23	31.2-72.0	23/25
Osun	25	25	33.1-65.8	All
Oyo	25	24	29.7-71.3	24/25
Ekiti	25	22	36.2-66.2	22/2

Key: AFM₁= Aflatoxin M₁, NAFDAC= National Agency for Food and Drug Administration and Control, EC= European commission, Codex= Codex alimentarius commission.

Discussion

The fungal isolates in this study included *Aspergillus flavus*, *A. fumigatus*, *A. terreus*, *A. parasiticus*, *A. niger*, *A. glaucus*, *A. versicolor*, *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp. and *Mucor* spp. The presence of species of *Aspergillus*, particularly *Aspergillus flavus* in this study could be attributed to the prevalence of their spores in the atmosphere. This organism was easily trapped during repackaging of the milk powder in the open markets. Since most fungal spores are found in the air, the spores must have contaminated the milk powder at different stages, such as: during opening of

bags, using unclean cups or containers for measurement, polluted environment, unhygienic handling by retailers, unclean/unsterilized packaging materials. The liberated spore can easily settle on food and ceilings of room and then germinate (Adebayo-Tayo *et al.*, 2009; Okhuoya and Ayanlola, 1986). Studies have shown that *Aspergillus* occurred highest in the number of colonies identified from air spora of some localities (Ilondu and Iloh, 2007; Dongo and Ayodele, 1997). *Aspergillus* spp. have been implicated in causing mycetoma in human (Cheesbrough, 2000). *A. flavus* is involved in allergic aspergillosis (pulmonary aspergillosis)

and also produces aflatoxin that is highly carcinogenic (Makun *et al.*, 2010; Prescott *et al.*, 2005).

Aflatoxins M₁ and B₁ in the locally repacked milk powder were analyzed using immunoaffinity column and high-performance liquid chromatography (IAC/HPLC) technique which is reported by many scientists as a sensitive technique. In addition, AFM₁ was analyzed using Aflasensor dipstick which is a simple, quick and inexpensive procedure for AFM₁ analysis. Findings revealed IAC/HPLC to be more sensitive than the Aflasensor kit with detection level of 32.8% for AFM₁ in the locally repacked milk powder compared to 53.6% by IAC/HPLC. Aflatoxin M₁ contamination of milk and milk products is a global problem and Nigeria is not excluded as this study shows 67 AFM₁ contaminated samples out of 125 (53.6%). Garrido *et al.* (2003) reported a 79.9% contamination of milk samples by AFM₁ collected from Brazil, at levels between 0.05 and 0.2 µg/kg. The AFM₁ concentrations (0.02-0.46 µg/kg) found in marketed locally repacked powdered milk in this study were all below the international regulatory limit (0.5 µg/kg) and this might be because the milk samples were all imported prior to repackaging and so compliance to the limit must have been achieved right at the point of manufacture. The health hazards associated with AFM₁ have been widely reported (Oluwafemi, 2012; Makun *et al.*, 2010; Adebayo-Tayo *et al.*, 2009). Of public health importance is the fact that the toxin is a potent carcinogen. Based on the findings in this work, the occurrence of AFM₁ does not appear to be serious public health hazard in Nigeria with regards to the locally repacked milk powder, probably due the fact that AFB₁ in the locally repacked sample must be converted to AFM₁, its dietary residue, after consumption (Makun *et al.*, 2010; Peraica *et al.*, 1999; Gbodi and Nwude, 1988).

On the contrary, AFB₁ occurred at unsafe levels in the locally repacked milk powder in this study, only awaiting consumption by human to be converted to AFM₁. This AFB₁ contaminated

locally repacked milk powder are usually consumed by the populace, especially the down trodden whose purchasing powers are low, probably due to the difficult economic situation in the country coupled with ignorance. This AFB₁ contaminated locally repacked milk powder are utilized in various ways: baking, fortification of meals such as cereal gruels (ogi), beverages, tea and sometimes direct oral consumption by children and young adults, including expectant mothers and the elderly. AFB₁ has been reported at high levels in locally produced soy milk powder under market condition in Nigeria (Adebayo-Tayo *et al.*, 2009). The incidence and concentration of AFB₁ found in the locally repacked milk powder samples were more elevated than those reported by Adebayo-Tayo *et al.* (2009) in locally produced soy milk powder in South-Southern Nigeria. This may be due to variations in ecology and climate of the South-West and South-South. Fungal growth and toxin production are dependent on climate and storage conditions and therefore vary with locations, with hot and humid climates and storage condition. The hot and humid conditions of South-West are more favourable for fungal growth and mycotoxins production than the cold condition in the South-South of Nigeria (Makun *et al.*, 2010).

Aflatoxin B₁ concentration (29.7-79.4 µg/kg) in the locally repacked milk powder analyzed were above the National Agency for Food and Drug Administration and Control (NAFDAC), European commission and codex (5 µg/kg) tolerance level for aflatoxin B₁ in food for human consumption.

Chronic consumption of this locally repacked milk powder with high concentrations of AFB₁, have deleterious public health implications such as immunosuppression, nephrotoxic and hepatocarcinogenic effects (Gbodi and Nwude, 1988; Peraica *et al.*, 1999; Makun *et al.*, 2010). Therefore, this practice of locally repacking powder milk for sale should be discouraged as it predisposes the wholesome product to fungal and mycotoxin contamination with consequent public health risk.



Figure 2: Aflasensor kit used for detection of aflatoxin M₁



Figure 3: Heat sensor for incubation



Figure 4: Negative and Positive control standard

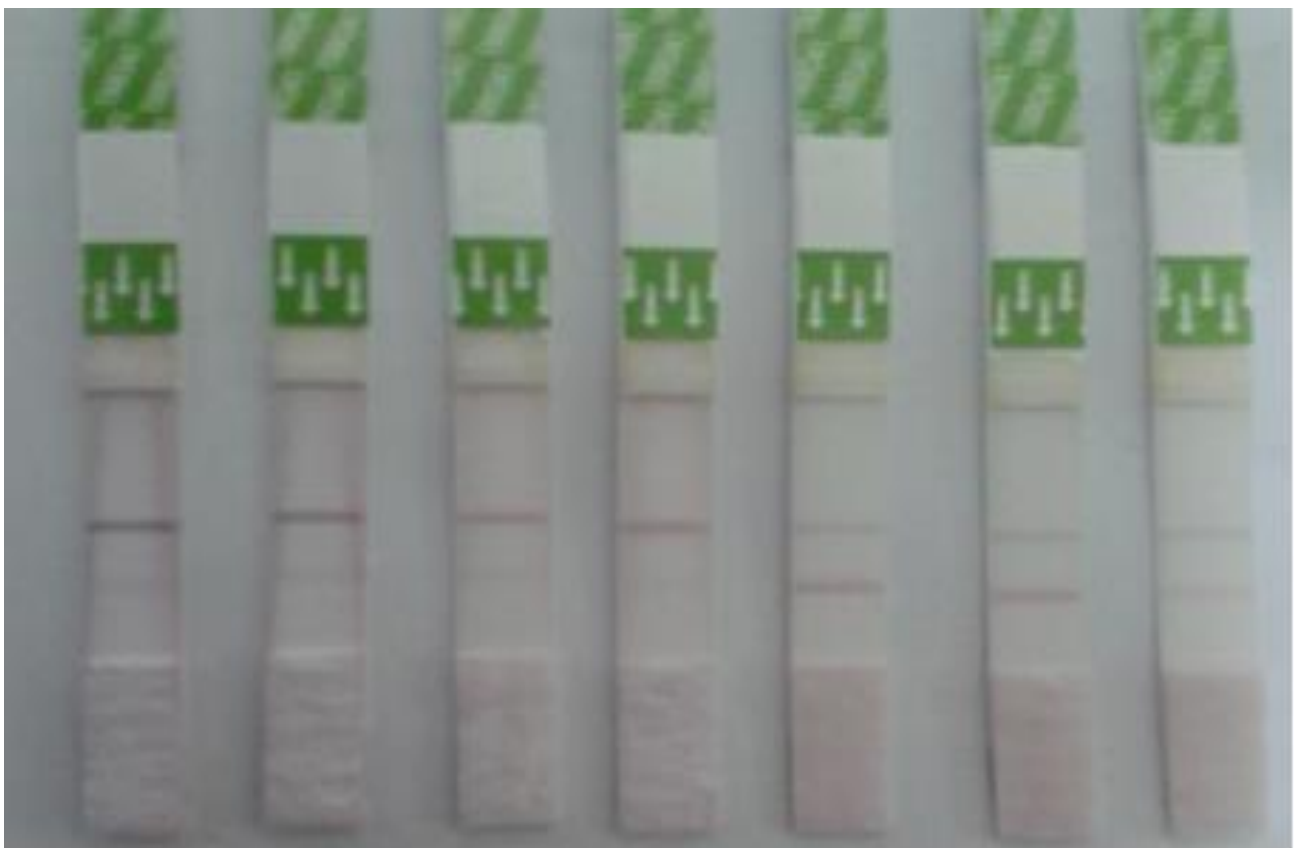


Figure 5: Valid tests for Aflasensor



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Citation: Oyeyipo, O.O., Oyeyipo, F.M. and Ayah, I.R. (2017). Aflatoxin (M_1 and B_1) Contamination of Locally Repacked Milk Powder in South-Western Nigeria. *Sokoto Journal of Medical Laboratory Science*; 2(3):174 – 187.

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