EXPANDING EXPORT OF SESAME SEED AND SHEANUT/BUTTER THROUGH IMPROVED SPS CAPACITY BUILDING FOR PUBLIC AND PRIVATE SECTOR

DEVELOPMENT OF A SIMPLE PREDICTIVE MODEL FOR MOULD GROWTH AND AFLATOXIN PRODUCTION IN THE NIGERIAN SHEA-NUT/ BUTTER PRODUCTION CHAIN

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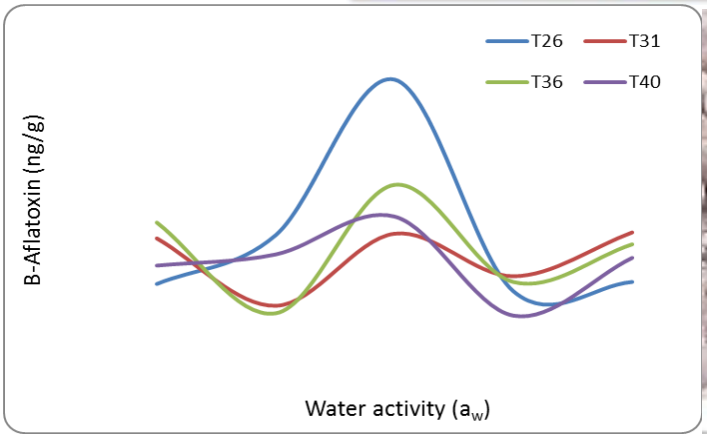

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1.1. INTRODUCTION

Aflatoxins are secondary metabolites produced by specific strains of *Aspergillus*. These species contaminate various agricultural commodities either before harvest or at post-harvest stages under favourable conditions of temperature and humidity. *Aspergillus* is a large genus of mould which grows at an optimal range of temperature of 28-33°C and at the water activity of about 0.83-0.97. The aflatoxigenic moulds, namely *A flavus*, *A parasiticus* and *A nomius* are principally found in soils and decaying vegetation. They occur in warmer parts of the world such as tropical region where temperature and moisture are high. They have a higher affinity of growth in nuts and oilseeds.

Aflatoxin contamination has been observed in a variety of agricultural commodities, including oil seeds and nuts. The recurring problem of mouldiness of Shea-nuts/kernels and high FFA and impurities in Shea butter due to the lack of established Hazard Analysis and Critical control points (HACCP) in the Nigerian Shea nut/butter production chain. The hydrolysis of fat is due to enzymatic action (fruit lipases and microorganisms' lipases). Boiling the nuts can inactivate the enzymes and the microorganisms can be suppressed by rapid and adequate drying (FAO, 1991). The current market standards preferred for Shea-kernels are: FFA (6%), fat content (45-55%), water content (<7%) and impurities (<1%). For Shea butter, the specified standards are; moisture (0.01-1.0%), FFA (3.5-4.0), acid value (1.0-10-0) and the colour should not be dark (Mensah, 2010). FFA in Shea butter is a function of hydrolysis of fat and produces rancidity and odours in the butter. FFA level is used as a standard for Shea butter export. When FFA values are above 2.0 mgKOH/g in Shea butter for export in Ghana, it is considered to be below standard for grade A Shea butter (Ayemibo, 2011).

Mould infestation and consequent aflatoxin contamination is more serious in the tropics due to the high temperature and humidity levels and the seasonal rainy seasons. High levels of aflatoxins have been found in groundnuts; up to 5000 µg/Kg (Kumar *et al*., 2008), Brazil nuts; up to 15 µg/Kg of G_1 (Oslen *et al*., 2008), Shea-nuts; up to 600µg/ (Kershaw, 1982), and up to 45 µg/Kg in sesame paste (Feng-Qin-Li *et al*., 2009). However, no work has been done to determine the relationship between mould growth and aflatoxin production through the Shea-butter value chain under varying conditions of water activity, relative humidity and temperature. The occurrence of food contaminants, mainly the mycotoxins, e.g. the aflatoxins is together with labelling and packaging deficiencies the main causes of notifications and/or exports rejections.

1.2. MATHEMATICAL MODELLING OF MOULD GROWTH AND AFLATOXIN PRODUCTION

Obviously, preventing fungal growth is the best method for avoiding toxin production consequently, control measures are directed towards factors that influence mold growth, such as water activity (moisture content), relative humidity, temperature, availability and composition of substrates, and presence of inhibitors. If contamination cannot be prevented, a way to either remove or destroy the aflatoxins will allow consumption of the commodity with reduced adverse effects.

Mathematical modelling of mould growth and toxin production under different physical conditions (temperature, relative humidity, pH and water activity) has been a useful tool in predictive mycology. This has been used to predict the extent of mould growth and invasion in foodstuffs as a function of environmental conditions. Mould growth and aflatoxin production as well as other mycotoxins have in recent times been modelled. Vaamonde *et al*., (2006) worked on the effect of water activity and temperature on production of aflatoxins and cyclopiazonic acid by *A. flavus* in pea-nuts. Monila and Giannuzi (2002), were able to model aflatoxin production by *A. parasiticus* in a solid medium at different temperatures, pH and propionic acid concetrations. Also, growth of *Fusarium langsethiae* and consequent T2 and HT-2 toxin contamination of oats under different growth conditions has been modelled by Mylona and Magan (2011).

Modelling mould growth in corn (Samapundo *et al*., 2007), considered the individual and combined effects of water activity and temperature on the radial growth of *A. parasiticus* and *A. flavus* on corn. Likewise, Galatia and Giannuzia (2011) conducted similar studies on Argentinian flint maize.

However, modelling of aflatoxin production in Shea butter production chains is a novel area and the result will help to establish the hazard critical control points in the value chain of the crop. Hence the objectives of this study are to:

Conduct a survey to identify the existing stages of Shea butter production and collect samples of Shea kernel/butter at the different identified stages from the major Shea-nut-producing states in Nigeria.

To identify moulds associated with different stages of Shea production chain.

To determine the relationship between water activity, temperature, relative humidity and aflatoxin production in Shea-nut production chain in order to establish the critical control points.

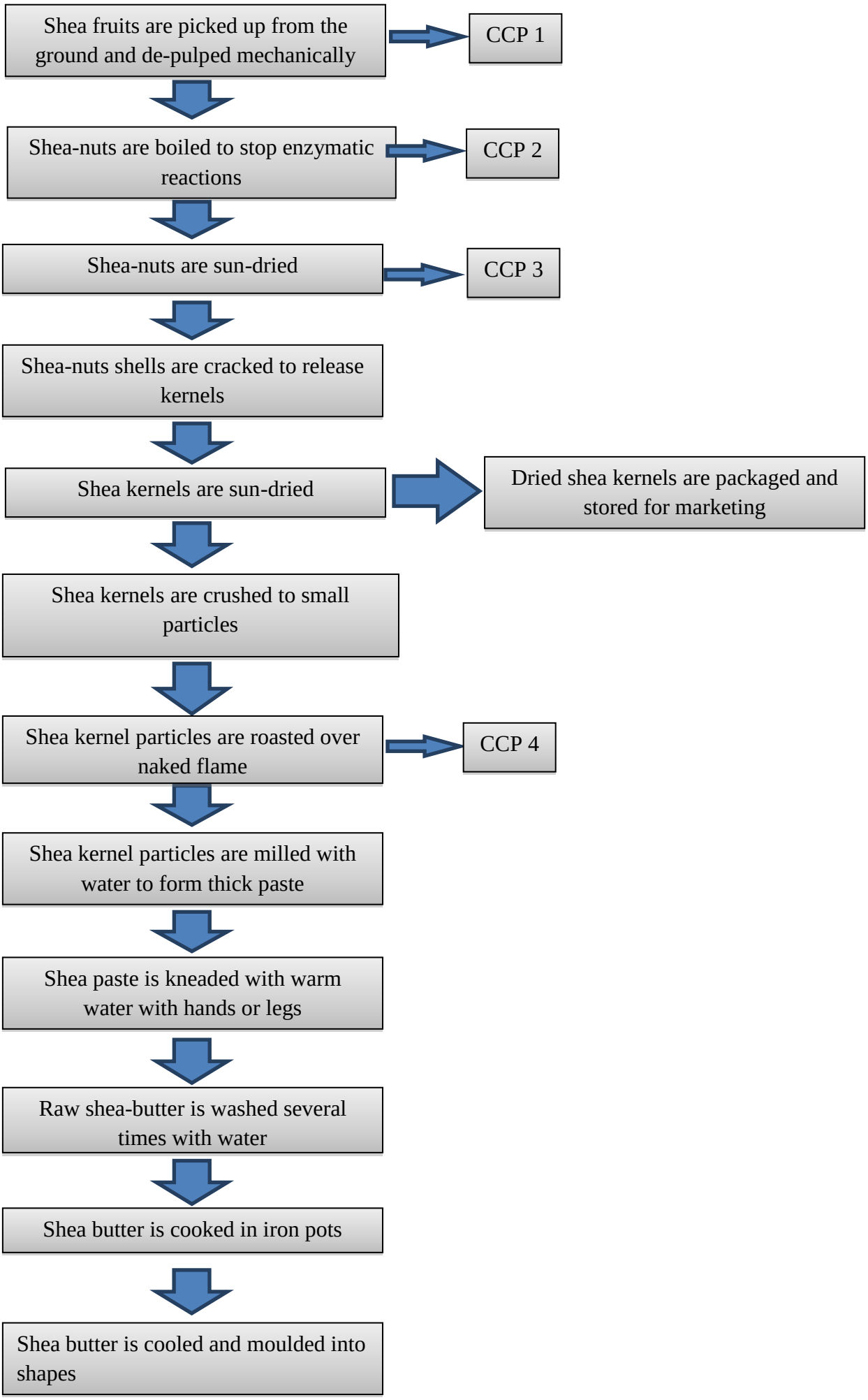
To develop a predictive model for aflatoxin production in the Shea production chain.

1.3. MATERIALS AND METHOD

1.3.1. Survey and sample collection of Shea nut and sesame in Nigeria

A survey was conducted in 3 Shea-producing states in Nigeria (Niger, Kwara, Oyo States) and samples were collected from at least 3 locations in each of the States. About 2-5 Kg sample of the 5 major production stages of Shea butter: fresh-in-shell nuts (FIS) n=9, boiled-in-shell nuts (BIS) n=4, dried kernels (DK) n=10, roasted-crushed-kernels (RCK) n=7 and Shea butter (SB) n=10 was collected from each of the following locations: Bosso, Etsu Audu, Dogongari, Karabunde (Niger State) Nuku, Kaiama, Agbaku-eji (Kwara State), Kisi, Igboho, Igbeti (Oyo State). A total of 40 Shea samples were collected and kept at 4 ºC. The flow chart for Shea butter processing is shown in Fig. 1. The temperature and relative humidity at the time of sampling was collected using a Thermo Hygrometer.

Fig. 1. Flow chart for the traditional method of Shea-butter production.



1.3.2. Sample preparation:

One hundred grams of each sample was weighed out and milled in a laboratory blender (Waring Commercial Laboratory Blender). The milled samples were kept in sterile sample bags and stored in the cold room. These sub-samples were used for isolation and colony forming unit (CFU/g) counts, aflatoxin analysis and determination of the free fatty acid content of the samples.

1.3.3. Isolation of Aspergillus spp from samples

The Isolation of *Aspergillus* was carried as described by Atehnkeng *et al.,* (2008). Where 1 g of each ground sample was aseptically (in a laminar flow hood) weighed into 10 ml sterile distilled water in a 40 ml sterile vial. This was mixed on a vortex shaker for 1 minute. 50, 100, 150, 200 and 500 µl were each plated on Modified Rose Bengal Agar (MRBA) medium (a seletive medium for *Aspergillus spp,* composition: (0.3% sucrose, 0.3% $NaNO_3$, 0.075% KH_2PO_4, 0.025% K_2HPO_4, 0.05% $MgSO_4.7H_2O$, 0.05% KCl, 1% NaCl, 1.3% EM agar, Rose Bengal, Dichloran, chloramphenicol and streptomycin). The inoculated plates were incubated upright at 31 ºC for three days.

1.3.4. Enumeration of Aspergillus species on clean up (MRBA) medium

The enumeration of *Aspergillus* colonies with distinct characteristics on MRBA medium was performed using a colony counter for each of the volumes plated, and the Colony Forming Units (CFU/g) estimated. Petri Plates with uncountable number of colonies were further diluted and plated again. During transfers of isolates, only plates with less than 10 colonies were transferred and all the colonies were transferred to avoid bias. Finally 20 isolates were picked up in each of the samples.

1.3.5. Identification of Aspergillus isolates

Aspergillus isolates were identified macro-morphologically by plating on 5/2 agar medium. After 5 days of incubation (unilluminated, 31 °C), isolates were classified into species and strains by observing colony characteristics, and conidial morphology as described previously (Cotty, 1989). Isolates that produced small sclerotia (average sclerotial diameter < 400 µm) on 5/2 were identified as having the S morphology (i.e. as being either S_{BG} or S-type *A. flavus*), while those with a smooth conidial surface and either an average sclerotial diameter > 400 µm or without sclerotia were identified as L-type *A. flavus.* Isolates that had dark green colonies on 5/2 and produced rough conidia were considered *A. parasiticus.*

1.3.6. Determination of free fatty acid (FFA) content of samples

The determination of FFA was carried out according to the method descibed by IOCCC (1996) in crude oils. Five grams of oil extract were melted and dissolved in 50 mL of diethylether and 95% ethanol mixture (1:1, v/v) and titrated against standardized 0.1 N alcoholic NaOH solution, using 1 mL phenolphthalein as indicator. A faint pink colour that persisted for 15 seconds indicated the end-point.

FFA (oleic acid %) was calculated using the formula below:

FFA (Oleic acid %) = 0.564 x V

where , Vt = volume of NaOH used in titration

1.3.7. Determination of total aflatoxins in Shea samples

Extraction and purification of total aflatoxins in samples was carried out by the BF-method for aflatoxins in peanuts and peanut products AOAC, (1990) with slight modifications using high performance thin layer chromatography (HP-TLC). Shea samples were extracted with 100 ml methanol/water (80:20 v/v) and 40 ml n-hexane. The samples were blended at high speed for 3 minutes. This was shaken for 30 minutes and filtered through Whatman no 1 filter paper. Purification of aflatoxins was done with 10% NaCl, and 30 ml n-hexane and finally into 25 ml dichloromethane. The extracts were allowed to dry in plastic cups and detection of aflatoxins was done by spotting on HP-TLC plates and developed in diethyl ether/methanol/water (96:3:1). Aflatoxins were quantified using a scanning densitometer and accompanying software (TLC Scanner 3, with Wincats 1.4.2 software, Camag, Muttenz, Switzerland). The minimum detection limit was 0.1 ng/g aflatoxin and the recovery rate was 87.6%.

1.3.8. Measurement of water activity of Shea samples

Water activity of samples was determined using isopiestic method decribed by Sablani *et al.,* (2001) using saturated salt solutions. In this method, 1.6 g of starch (standard material) was dried in the oven at 70 ºC for 24 hours using $CaCl_2$ as the absorbent. Starch standard was equilibrated with saturated salt solutions: KI, KCl, NaCl, $NaNO_3$, $(NH4)_2SO_4$, and $MgCl_2$ in dessiccatiors at 31.52 ºC to establish different relative humidities which was measured with Hobo data loggers. After equilibration, (usually after 12-25 days) the moisture contents of the standard over the different salt solutions was determined by halogen moisture analyzer. A graph of of moisture content and Equilibrium relative humidity (water activity) of salt solution was plotted and the standard curve was modelled by the GAB equation (Bell and Labuza, 2000). Same mass of standard was then

equilibrated over 20 g of sample in a dessicator. The moisture content of the standard was determined after 48 hours. The water activity of the samples was estimated from the standard curve. Water activity of standard is equal to water activity of sample at equilibrium.

1.3.9. Rehydration of Shea kernel and determination of water activity levels for the modelling activity

Ten grams of slightly crushed Shea-kernels (appx. 6 mm x 5 mm) was weighed into 30 ml vials and sterilized at 121^{o}C for 20 minutes to kill all spores and organisms that may be present in the kernels. Different volumes of sterile water (100 to 3000 µl) were added to the sterile samples and were thoroughly mixed on a vortex mixer to obtain a homogeneous mixture. The vials were stored at 4 ^{o}C for 72 hours for equilibration to take place. The moisture contents (MC) of the equilibrated samples was thereafter determined by the oven method at 103 ^{o}C. The MC values obtained were fitted into the absorption isotherm curve of Shea-kernels, developed by Kapseu *et al*., 2006, which was modelled using the GAB equation (Bell and Lazuba, 2000) to obtain the corresponding water activity values of the hydrated kernels. The water activity levels chosen for inoculation are 0.887, 0.907, 0.95 and 0.985, and MCs for each of these water activity levels were appx. 17%, 18.5%, 22.4%, and 26.3%, respectively..

1.3.10. Inoculation and incubation of Shea-kernels

Ten grams of autoclaved Shea kernel samples in vials with known water activity levels were inoculated with 500 µl of 10^6 spore suspension of toxigenic *A flavus L,* previously isolated from the samples and confirmed to be toxigenic. The inoculation was replicated 4 times for each water activity level. The inoculated samples at the 4 water activity levels were incubated at 25, 31, 36 and 40 ^{o}C along with an un-inocualted control sample for each water activity level. The samples were incubated over glycerol/water solutions, adjusted to the water activity levels of the samples inside sealed dessiccators/polythene bags for 7 days. Samples at water activity zero aw (dry state) were also inoculated and incubated along with the hydrated samples at the 4 temperature regimes.

1.3.11. Enumeration of fungal spores on inoculated Shea nut samples after incubation

After 7 days of incubation, of the inoculated Shea samples colonization by A flavus at different temperature and water activity levels, was estimated by counting the number of spores of *A. flavus* with a turbidimeter. Samples with high spore concentrations were diluted appropriately for accurate estimation of the number of spores.

1.3.12. Extraction and quantification of total aflatoxins in inoculated Shea samples

Ten grams of inoculated sesame samples was extracted with 50 ml 80% methanol and 2 5 ml n-hexane, which were blended at hi-speed for m minutes, shaken for 30 minutes and filtered with Whattman no 1 filter paper. The extract was further purified with h-hexane and NaCl solution and toxins were partitioned into 25 ml dichloromethane. The dried extract was spotted on HP-TLC plates and quantified using scanning densitometer, CAMAG TLC Scanner 3 with win-CATS 1.4.2 software (Camag AG, Muttenz, Switzerland), as described previously (Suhagia *et al*., 2006).

1.3.13. Statistical analysis

Data on fungal incidence aflatoxin production model was summarized and analyzed using SAS (version 9.1, SAS Institute Inc., Cary, NC) and means were separated using Fisher's protected least significant difference (LSD) test to determine significant differences among the samples Means for Aflatoxin levels, free fatty acids, degree of colonization were separated using descriptive statistics.

Polynomial equations for the amount of spores produced as well as toxin production with regard to the incubation at different temperatures and water activity were obtained by forward stepwise regression using the SAS 9.2 package. These equations included both linear effects of a_w and temperature as well as their interactions.

1.4. RESULTS

1.4.1. Survey to collect Shea nut samples

The different locations where Shea samples were collected are shown on the Nigerian map (Fig. 2) with purple dots. The relative humidity at the time of the sampling ranged from 51.8% in Etsu-Audu in Niger State to 81.9% in Dogongari, Niger State. Temperature also ranged from 26.0 ºC for Kisi in Oyo state to 40.6 ºC in Karabunde in Niger State (Table 2).

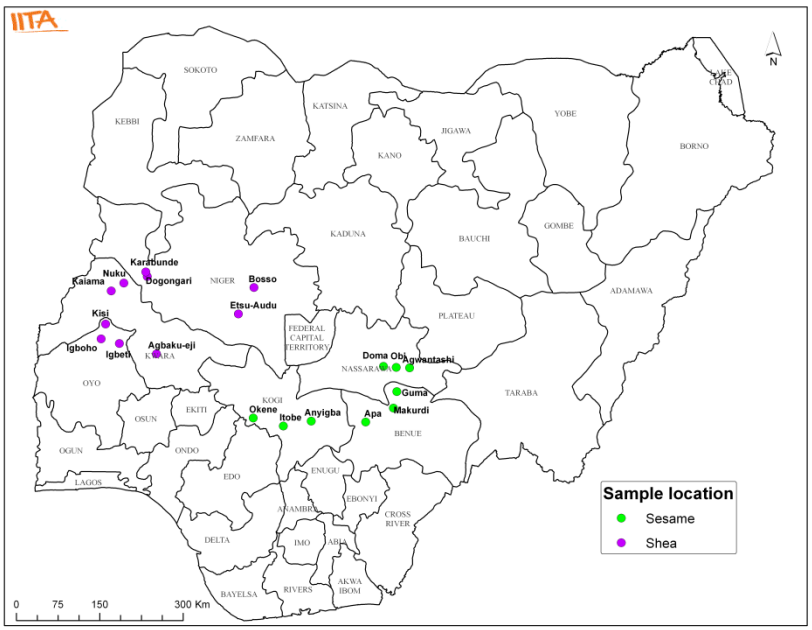


Fig. 2. Map of Nigeria showing locations where the Sheanut and sesame samples were collected

Table 2. Locations visited during survey and number/type of Shea samples collected

State	Location	No. of Sample	Type/Variety of Sample Collected	RH (%)	Temp.(°C)
Niger State	Bosso	5	FIS; BIS; RCK; DK; SB	60.1	33.6
	Dogongari	3	FIS, BIS, DK, SB	81.9	25.9
	Etsu-Audu	4	FIS, DK, RCK, SB	51.8	35.9
	Karabunde	4	FIS, DK, RCK, SB	54.9	40.6
Oyo State	Kisi	4	FIS, DK, RCK, SB	79.2	26.0
	Igboho	3	FIS, DK, SB		
	Igbeti	2	DK, SB	68.0	29.9
Kwara State	Kaiama	4	FIS, BIS, DK, RCK, SB	66.8	31.4
	Nuku	4	FIS, DK, RCK, SB	76.2	30.6
	Oke-oyi	5	FIS, BIS, DK, RCK, SB	67.9	29.5

FIS – Fresh-in-shell nuts; BIS – Boiled-in-shell nuts,
RCK – Roasted crushed kernels; DK – Dried kernels; SB – Shea butter.

1.4.2. Sources of contamination of Shea nuts/kernels in the field and during storage

The quality of Shea butter after processing depends on the quality of the nuts used for processing. Good quality nuts, which are picked up immediately when they drop from the tree, and then dried and processed, will give a better quality of Shea butter. However, Shea nuts that are abandoned in the field for a long time are likely to decompose and are colonized by aflatoxigenic fungi (Fig 2).

Inadequate drying of Shea kernels to safe moisture level before storage, poor hygienic condition, rewetting and poor aeration, can enhance colonization by aflatoxigenic fungi, leading to aflatoxin contamination in Shea kernels. In Shea, the processing stages are many, and the traditional methods involve drying nuts on bare ground, the use of one container for several other processes, and kneading with legs and hands, giving room for contamination by aflatoxigenic moulds (Fig 3). The different stages of Shea processing and the critical control points are described in Table 3.

1.4.3. Distribution of Aspergillus species in Shea samples

Generally*, Aspergillus flavus L* was the most predominant isolate in Shea samples in all the states.

A.flavus L occurred in all the production stages; however, the occurence is significant in dried kernel P<(0.05) samples across the three states, which may be the reason for high levels of aflatoxin B_1 at this production stage (Table 4). The occurrence of *Aspergillus* species was lowest in Shea butter in all the states compared to dry kernels. This shows the possibility of toxins being distributed across the production chain from fresh nuts to the Shea butter, which is the end product. Other *Aspergillus* species like *A. tamari, A. niger, A. tereus*, and *A. fumigatus* were observed, but they are not of significance because they are not implicated in aflatoxin production.

Fig. 2. Shea nut on the tree (A) or freshly harvested (B, C) are likely to have little of no fungal contamination, but nuts abandoned on the ground (D, E, F, G, H, I, J, K) are likely to have been colonized by aflatoxin producing fungi

Fig. 3:. Processing methods such as drying of depulped Shea nuts on bare ground (A), poor hygienic conditions during roasting of the nuts and milling (B, C), kneading milled roasted kernels with legs and hands (D, E), poor storage conditions of the knealed kernels (F, G), cooked/boiled Shea buttter (H) and moulded Shea butter (I) could lead to contamination by aflatoxigenic fungi and consequently, the production of aflatoxins

Table 3: Different stages of processing and Critical Control Points (CCP) in the Shea-nut/butter production chain

Stage of Processing	Practices/Purpose	Possible risk	Preventive measure	CCP
Collection of fallen Shea fruits	Picking up fruits several days after they have fallen to the ground in order to gather a size-able quantity	Decomposition of fruits by spoilage microrganisms that eventually infect nuts	Fallen fruits should be picked up for processing immediately	CCP
De-pulping of fruits	Piling of fruits in a heap to decompose naturally, mechanical removal of pulp with hand or by eating pulp or boiling to soften pulp	Piling will lead to fermentation of sugars in pulp, which may affect kernel quality and produce rancid odours in butter	Fruit pulp should be removed mechanically, immediately after collection and boiled	
Boiling of fresh nuts	Boiling of nuts in water in clay/metal pots over naked flame to de-activate enzymatic activities in the nuts/kernels	If boiled nuts are not dried rapidly and effectively, mouldiness of nuts results	Boiling of nuts should be carried out immediately after de-pulping for 1-2 hours before drying	CCP
Drying of boiled nuts	Drying on bare surfaces like roadside, compound floor, rock surface	Infection of nuts and kernels by toxigenic moulds from the soil and air, which lead to mouldiness and high FFA values	Use of driers	CCP

Stage of Processing	Practices/Purpose	Possible risk	Preventive measure	CCP
Cracking of nuts	Cracking of dried nuts with stone to release kernels	If nuts are properly boiled and dried, nuts will be cracked easily without damage to the kernels		
Drying of Shea kernels	Drying on bare ground, rock surfaces and roadside	Infection by toxigenic moulds from surfaces, air and animal droppings, leading increased mould load, toxins and FFA values	Use of driers that will dry kernels to a safe moisture level	CCP
Roasting of crushed kernels	Roasting of crushed kernels over naked flame to enhance butter production	Introduction of poly-cyclic aromatic hydrocarbons –PAH from wood smoke into the Shea butter, some of which are confirmed carcinogens	Use of roasters	CCP
Grounding of roasted and crushed kernels	Grounding with water to form a thick paste			
Mixing/kneading of the resulting paste	Kneading with hands and legs in order to lighten paste and get butter floating	Contamination of butter by microorganisms from the skin and dirty water used for mixing	Use of kneading machines and clean water	
Boiling of raw Shea butter	Boiling in metal pots to cook butter			
Moulding of Shea butter	To form shapes			

Table 4. Distribution of *Aspergillus* isolates in Shea samples collected at different processing stages in some states in Nigeria.

State	Type of Sample	No. Isolated	% of Fungal species				
			A. flavus	*A. Tamarii*	*A. niger*	*A. tereus*	*A. fumigants*
Niger	FIS	66	69.7	0.0	4.6	22.7	3.0
	BIS	40	100.0	0.0	0.0	0.0	0.0
	RCK	50	42.0	6.0	48.0	4.0	0.0
	DK	127	74.0	6.3	18.9	0.8	0.0
	SB	47	42.6	0.0	57.5	0.0	0.0
Oyo	FIS	11	27.3	0.0	72.7	0.0	0.0
	RCK	7	28.6	0.0	71.4	0.0	0.0
	DK	102	65.7	2.0	32.4	0.0	0.0
	SB	8	50.0	0.0	50.0	0.0	0.0
Kwara	FIS	34	88.2	0.0	8.8	0.0	2.9
	BIS	53	66.0	0.0	33.9	0.0	0.0
	RCK	33	30.3	0.0	69.7	0.0	0.0
	DK	162	74.1	3.7	22.2	0.0	0.0
	SB	26	30.8	0.0	69.2	0.0	0.0
			27.8	8.4	6.8	5.2	2.0

LSD (P = 0.05)

FIS – Fresh-in-shell nuts ; BIS – Boiled-in-shell nuts ; RCK – Roasted crushed kernels ; DK – Dried kernels; SB – Shea butter; LSD- Least significant difference = 0.05

1.4.4. Aflatoxin concentration in Shea samples

Aflatoxin levels were observed at all the stages of production in Niger state with the highest at the roasted crushed kernel. The least (0.2 ng/g) aflatoxin concentration was obtained in fresh-in-shell nut and boiled-in-nut (1.02 ng/g) samples with a range of 0.0 to 1.9 ng/g. The highest aflatoxin concentration was obtained from DK samples with the mean concentration of 3.4 ng/g in both Niger and Oyo states, with a range of 0.0 to 6.0 and 2.0 to 5.9 respectively, while Kwara had a mean concentration of 2.5 ng/g. High levels of aflatoxin concentration (2.9 ng/g) was also observed in roasted-crushed-kernels from Niger State, with a mean range of 0.0 to 8.8 ng/g compared to Oyo and Kwara (Table 4). The high concentration of the aflatoxin obtained in these samples at different stages, correlates with the high frequency of *Aspergillus* species in each of the stages.

1.4.5. Colony forming units of Shea nut samples

The CFU count of roasted-crushed-kernels (RCK) and Shea butter (SB) samples was low across the states sampled, probably because of heat treatment in the samples. The roasting process may have killed the fungal spores, though this may not cause a reduction in the amount of toxins, as the heat of roasting does not denature aflatoxins. However, dried kernels (DK) had the highest CFU count across the samples in all the states. This is attributed to poor dying conditions and contamination from extraneous materials that the kernels are normally subjected to during the drying process. Boiled-in-shell nuts (BIS) samples also had high CFU count across the states sampled, which can also be explained as being caused by slow and inadequate drying after boiling, which places the nuts in a more susceptible state for colonization by moulds (Table 5).

1.4.6. Free Fatty Acids (FFA) of Shea samples

The result of FFA, which is a function of fat hydrolysis by lipase enzymes produced in the fruit itself, and by moulds obtained for all the stages. The mean occurrence of FFA especially at the DK stage was generally above the permissible limits (3.5-4.0%) values for premium Shea butter for export. The occurrence of FFA in the SB product (2.2%) was lowest in Niger with a range of 0.3 - 4.2% compared to Oyo (5.8%) with a range of 2.9 – 11.3% and Kwara (3.7%) with a range of 1.9 – 6.3% (Table 6).

Table 5. Aflatoxin concentration (ng/g) in Shea samples collected at different processing stages in some states in Nigeria

Location/ State	Type of Sample		Aflatoxin concentration (ng/g)			
			B_1	B_2	G_1	G_2
Niger	FIS	Range	– 0.6	1.9 -5.6	0.0 – 2.7	0.0 – 0.0
		Mean (n=4)	0.2	1.9	0.7	0.0
	BIS	Range	0.8 - 2.0	0.4 - 0.9	0.0 - 0.0	0.0 - 0.0
		Mean (n = 2)	1.02	0.62	0.0	0.0
	RCK	Range	0.0 - 8.8	0.0- 1.6	0.0 - 0.0	0.0 - 0.0
		Mean (n = 3)	2.9	0.5	0.0	0.0
	DK	Range	0.0 - 6.0	0.0 - 3.1	0.0 - 0.0	0.0 - 0.0
		Mean (n = 4)	3.4	1.4	0.0	0.0
	SB	Range	0.0 - 2.5	0.0 - 0.9	0.0 - 2.3	0.0 - 2.1
		Mean (n = 4)	1.2	0.4	0.9	0.9
Oyo	FIS	Range	0.0 - 0.0	0.0 - 0.0	0.0 - 0.0	0.0 - 0.0
		Mean (n = 3)	0.0	0.0	0.0	0.0
	DK	Range	2.0 - 5.9	0.0 - 3.3	0.0 - 0.0	0.0 - 0.0
		Mean (n = 3)	3.4	1.5	0.0	0.0
	SB	Range	0.0 - 0.0	0.0 - 0.0	0.0 - 0.0	0.0 - 0.0
		Mean (n = 3)	0.0	0.0	0.0	0.0
Kwara	FIS	Range	0.0 - 1.9	0.0 - 0.6	0.0 - 0.0	0.0 - 0.0
		Mean (n = 3)	0.9	0.2	0.0	0.0
	BIS	Range	0.0 - 0.4	0.0 - 0.0	0.0 - 0.0	0.0 - 0.0
		Mean (n = 2)	0.2	0.0	0.0	0.0
	RCK	Range	0.0 - 0.0	0.0 - 0.0	0.0 - 0.0	0.0 - 0.0
		Mean (n = 3)	0.0	0.0	0.0	0.0
	DK	Range	1.1 - 3.3	0.4 - 1.2	0.0 - 0.6	0.0 - 0.4
		Mean (n = 3)	2.5	0.9	0.4	0.1
	SB	Range	0.0 - 0.0	0.0 - 0.0	0.0 - 0.0	0.0 - 0.0
		Mean (n = 3)	0.0	0.0	0.0	0.0

FIS – fresh-in-shell nuts, BIS – boiled-in-shell nuts, RCK – roasted crushed kernels, DK – dried kernels, SB – Shea butter. At the time of the survey, only samples at storage were available for sesame, while for Shea, samples were collected from at least four stages of production.
Aflatoxin concentration value 0 = non detectable; The minimum detection limit was 0.1 ng/g Aflatoxin; Recovery rate was 86.6%

Table 6: Colony forming units (CFU/g) of *Aspergillus spp* in Shea samples collected at different processing stages in some states in Nigeria

Location/ State	Type of Sample	No. of Samples	Mean CFU/g of *Aspergillus spp*	Range
Niger	FIS	4	3565	60 – 9600
	BIS	2	6650	6500 – 6800
	RCK	3	767	300 – 1600
	DK	4	7125	3900 – 10000
	SB	4	885	140 –1800
Oyo	FIS	2	900	600 – 1200
	DK	3	7000	1400 – 13000
	SB	3	143	80 – 250
Kwara	FIS	3	1733	600 – 4000
	BIS	2	18100	13500 – 23700
	RCK	3	333	200 – 600
	DK	3	22367	12900 – 36000
	SB	3	213	40 – 300

FIS – fresh-in-shell nuts, BIS – boiled-in-shell nuts, RCK – roasted crushed kernels, DK – dried kernels, SB – Shea butter, CFU/g = colony forming units/g of sample

Table 6: Free fatty acid content of Shea samples collected at different processing stages in some states in Nigeria

Location/ State	Type of Sample	No. of Samples	Mean FFA (%)	Range (%)
Niger	FIS	4	2.7	1.4 - 4.1
	BIS	2	3.7	0.0 - 0.0
	RCK	3	3.0	1.7 - 5.5
	DK	4	5.2	3.0 - 7.7
	SB	4	2.2	0.3 - 4.2
Oyo	FIS	2	2.8	2.3 - 3.3
	RCK	1	1.1	0.0 - 0.0
	DK	3	3.9	1.2 - 8.4
	SB	3	5.8	2.9 -11.3
Kwara	FIS	3	1.9	1.1 - 3.0
	BIS	2	3.8	0.0 - 0.0
	RCK	3	4.5	2.3 - 6.2
	DK	3	5.8	4.7 - 7.2
	SB	3	3.7	1.9 - 6.3

FIS – fresh-in-shell nuts, BIS – boiled-in-shell nuts, RCK – roasted crushed kernels, DK – dried kernels, SB – Shea butter, FFA = Free Fatty Acids

1.4.7. Measurement of water activity of the samples

A graph of moisture content and equilibrium relative humidity (water activity) of salt solution was plotted (Fig. 4, 5) and the standard curve was modelled by the GAB equation shown below

$$M_w = \frac{M_m\, YKa_w}{(1-Ka_w)(1-Ka_w + YKa_w)}$$

where M_m = Monolayer moisture (Kilogram water/Kilogram dry solid)

a_w = water activity

M_w = Moisture content (d.b)

Y; K; M_n = three free sorption parameters characterizing the sorption properties of the material.

The graph was then used to determine the water activities of the samples (Table 7).

Shea butter samples across the states had the least water activity values (0.223) as expected, hence the low mould count in the samples. Most storage moulds do not grow at a water activity level below 0.80; however their spores still remain on the substrate, in a passive state. Fresh-in-shell nuts had the highest water activity value (0.933) across the states, while Shea butter had the least water activity across the states. Samples with high water activity are likely to have more aflatoxigenic fungi than samples with low water activity. The level of water activity in each sample determines the degree of colonization by aflatoxigenic fungi, leading to aflatoxin production.

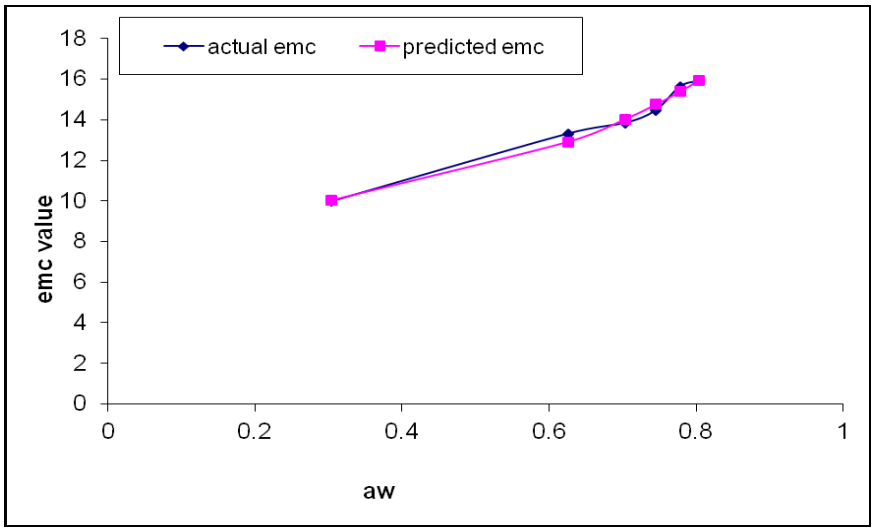


Fig 4: Graph of equilibrium moisture content versus water activity of standard, as modelled by the GAB equation: emc = equilibrium moisture content, a_w = water activity

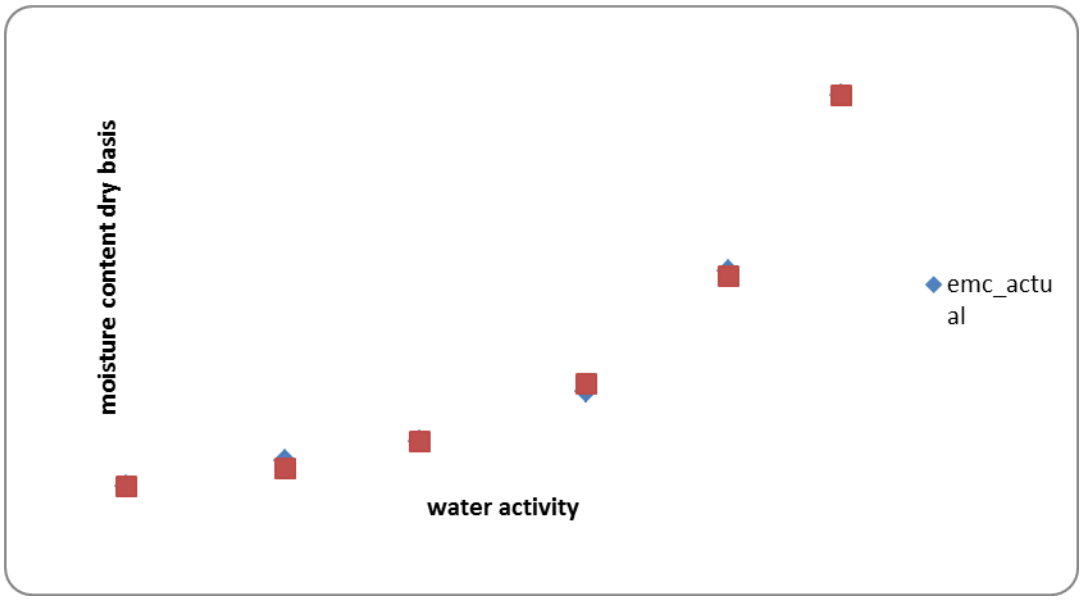


Fig 5: Graph of water sorption isotherm of Shea kernels, developed by Kapseu *et al*., (2006), modelled by the GAB equation to obtain the predicted water activity values

Table 7. Water activity (a_w) values of Shea samples collected at different processing stages in some states in Nigeria

Location/ State	Type of Sample	EMC (%)	ERH (%)	Water activity (A_w)
Niger	FIS	19.62	93.30	0.933
	BIS	18.99	91.50	0.915
	RCK	15.06	85.00	0.850
	DK	14.46	72.80	0.728
	SB	10.53	38.20	0.382
Oyo	FIS	18.89	91.20	0.912
	RCK	15.88	80.10	0.801
	DK	14.06	70.50	0.705
	SB	9.56	22.30	0.223
Kwara	FIS	18.66	90.50	0.905
	BIS	18.76	90.80	0.908
	RCK	11.39	48.70	0.487
	DK	14.76	74.50	0.745
	SB	10.18	33.00	0.330

FIS – fresh-in-shell nuts, BIS – boiled-in-shell nuts, RCK – roasted crushed kernels,
DK – dried kernels, SB – Shea butter.
EMC - Equilibrium Moisture Content, ERH - Equilibrium Relative Humidity, a_w - ERH/100

Predicting aflatoxin production for Shea nut

1.4.8.1. Effect of Temp (°C) and a_w on Shea fungal colony forming units (CFU) count

The experiment was to evaluate the effect of temperature and water activity on the growth and sporulation of *Aspergillus* species in Shea nut. SAS regression model was used to establish the relationship between water activity, temperature and CFU count in Shea kernels after inoculation with *A flavus L* and incubation for 7 days at 5 water activity levels and 4 temperature regimes.

Table (8) shows the logarithmic mean of *Aspergillus* CFU/g count at the 5-water activity levels over four temperatures:

High spore count of *A flavus L.* was observed from a_w 0.885 and remained relatively constant up to a_w 0.958, and started to reduce at the wettest condition of 0.993, which shows that this condition may have been too wet for proliferation of fungal spores Fig. 6A.

The lowest spore counts were observed in the base line samples, since the Shea kernels were very dry at this level. High CFU counts were also observed at temperatures 25-31 ^oC and gradually reduced at 36 ºC and then 40 ^oC Fig. 6B, which supports the fact that *Aspergillus flavus L* grows at an optimum temperature of 27-31 ^oC. This implies that water activity significantly influences the degree of *Aspergillus* colonization in Shea nut and subsequent aflatoxin formation.

Table 8: Parameter estimate ± S.E and corresponding p-values for stepwise regression analysis for the effect of water activity (a_w) and temperature on spore concentration

Variable	*Estimated value ± S.E*	*p value*	*aw^2t^2 dropped Est. value ± S.E*	*P value*	*aw^2 dropped est. value ± S.E*	*P value*	*aw.t dropped est. value ± S.E*	*p value*
Intercept	-33.588 ± 34.396	0.3369	-4.743 ± 6.988	0.5025	-3.139 ± 4.421	0.4829	-0.307 ± 2.491	0.9027
Aw	52.083 ± 52.527	0.3296	8.555 ± 13.254	0.5235	4.780 ± 4.019	0.2433	1.693 ± 0.624	0.0107
T	1.726 ± 1.415	0.2323	0.525 ± 0.189	0.0094	0.525 ± 0.187	0.0084	0.44 ± 0.15	0.0062
Awt	-1.402 ± 1.533	0.368	-0.093 ± 0.121	0.4495	-0.093 ± 0.119	0.4426		
aw^2	-15.275 ± 16.952	0.375	-2.194 ± 7.329	0.7667				
t^2	-0.017 ± 0.012	0.1475	-0.008 ± 0.002	0.0028	-0.008 ± 0.002	0.0024	-0.008 ± 0.002	t^2
aw^2t^2	0.012 ± 0.014	0.3987						

Intercept = constant, a_w = water activity, t = temperature, a_wt = water activity and temperature interaction, a_w^2 is the square of the water activity, t^2 is the square of temperature

From the SAS regression model for Shea spore count
log CFU = $-0.307 + 1.693a_w + 0.44t - 0.008t^2$
log CFU = $C_0 + C_1 .aw + C_2 .t - C_3.t^2$
where Cs are constants a_w is water activity and t is temperature in degrees Celsius

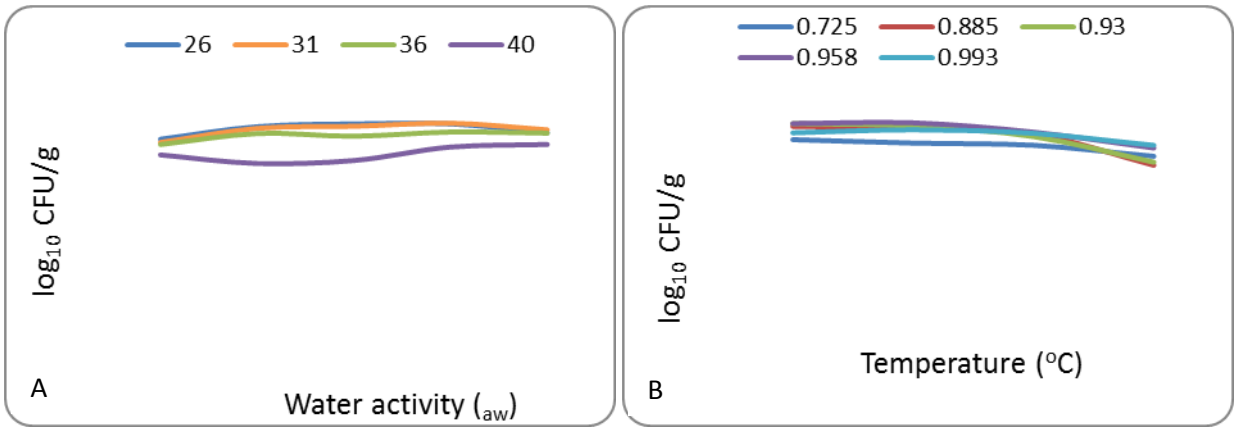


Fig. 6A: plot of $\log_{10}$ of fungal spores produced at 5 water activity levels and 4 temperatures (6B) after 7 days of colonization by *Aspergillus flavus L*

1.4.8.2. Effect of Temp (°C) and a_w on B-aflatoxin production in inoculated Shea kernel samples over a 7-day incubation period

Shea nut were dehydrated and subjected to different water activity and temperature. Figure. 7 shows the B-aflatoxins (B_1+B_2) produced in the inoculated samples over a 7-day incubation period over the 4 specified temperature regimes. At all water activity levels, the concentration of aflatoxin produced was generally low (Fig. 8). In this study, it was also observed that the amount of aflatoxin, produced at temperature 31-36 °C for all the a_w levels apart from 0.850 and 0.905, was constant, and then started to reduce at 40 °C. It can be said that temperature 31-36 °C and water activity 0.905 is the optimum for aflatoxin production in Shea kernels, even though they are in small quantities.

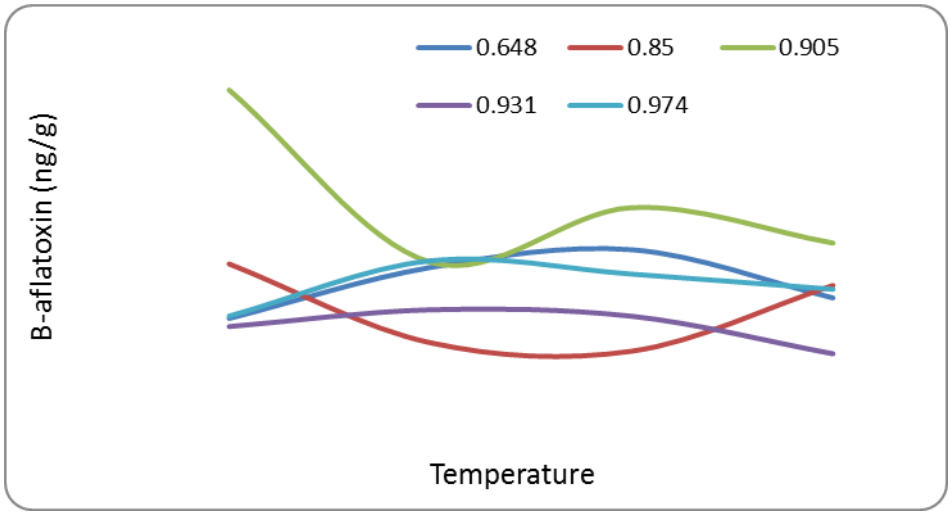


Fig 7: Graph of aflatoxin Σ (B1+B2) at five water activity levels and four temperature regimes

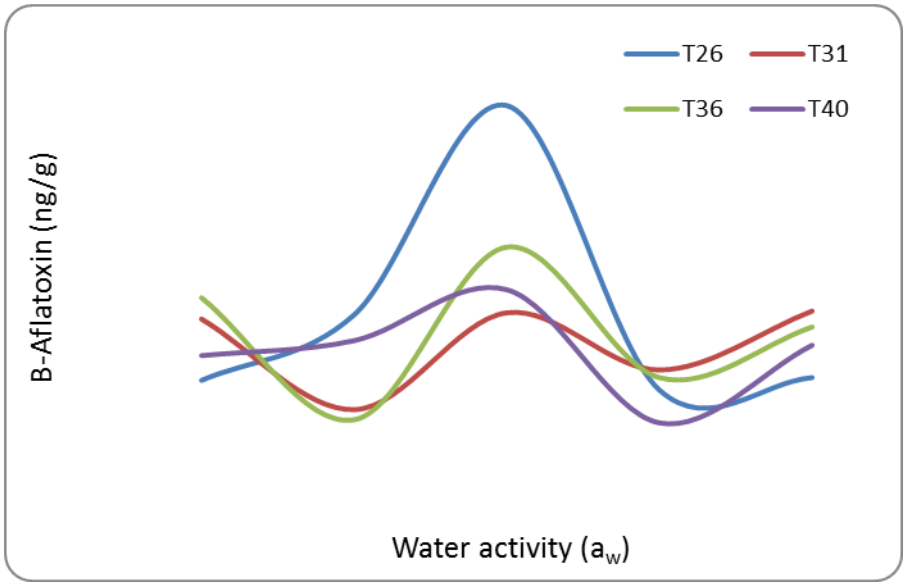


Fig 8: Graph of aflatoxin Σ (B_1+B_2) at four temperature and five water activity levels

1.4.8. The predictive equation for aflatoxin production in Shea

The aflatoxin data generated from inoculation with aflatoxigenic *A. flavus* strain at different temperatures and water activity was subjected to the SAS regression model to establish the relationship between water activity, temperature and CFU count in Shea kernels. The water activity and temperature had significant effect on colonization and spore concentration on Sheanut. However, the differences in spore concentration did not have significant effect on aflatoxin concentration. Thus a predictive equation for aflatoxin formation could not be determined because of the low concentration and variability of aflatoxin concentrations within and between the different temperature regimes and water activity.

1.5. DISCUSSION

This is the first study to observe the distribution of *Aspergillus spp* across the Shea-kernel/butter production chain and to study the possible effects of this distribution, such as FFA and aflatoxin contamination. The occurrence of *Aspergillus flavus L* as the predominant *Aspergillus sp* through the Shea value chain shows that contamination of Shea starts from the field when the fresh Shea fruits drop from the tree to the ground and are not picked up on time. When abandoned in the field for longer period *Aspergillus spp,* which are abundant in the soil, begin to decompose the fruit and subsequent accumulation of aflatoxins.

From this study the aflatoxin contamination values were generally low in the Shea-products as seen across the value chain. Aflatoxin levels across the stages in the 3 states were considerably low; only about 13.2% of the 40 samples collected had above 4 µg/Kg (ppb), which is the

acceptable limit for total aflatoxins in oil seeds and nuts in the EU countries. This result correlates with the work carried out by Kershaw (1982), who found aflatoxin levels in commercial Shea kernel samples collected from some selected countries in West Africa. A greater proportion of the samples (85.7%) had aflatoxin concentration $B_1 \leq 5$. Also, ICRAF (2000) identified an advantage that Shea cake has over other oil seed cakes like groundnut, cottonseed and palm kernel cake for livestock feed because of low aflatoxin content, even when mouldy (Ugese, 2010). However, the fact that aflatoxins occurs through all the processing stages, explains the possibility of aflatoxins being transferred from the raw materials to the finished product. All the DK samples in the 3 states were contaminated with low levels of aflatoxins, which explains the high *Aspergillus* CFU counts at this processing stage.

High CFU counts in the BIS and DK stages of processing indicate that the boiling process, which is necessary for de-activation of enzymes, such as the lipase enzymes among other advantages, should be followed by rapid drying of the nuts on a mat or raised platform and not on bare ground, or better still in solar/electric dryers. The complete drying process of the kernels to a safe moisture level (< 7%) should also be done rapidly to avoid contamination by microorganism. These microorganisms contribute to the mould load and consequently the FFA values, which are an important quality parameter for rating Shea kernels/butter into the different quality grades. The presence of FFA is similar to the findings of Nikiema and Umali, (2007), and ICRAF (2000).

Besides FFA, acidity, saponification value, peroxide value, and iodine value, which are dependent on how fast the nuts are picked up when they fall from the trees to the ground, the parboiling, the drying method and duration and processing technique (Aculey *et al*, 2012), should be given more attention.

From this study modelling the formation of aflatoxin in Shea nut can be difficult, because the chemical composition does not support the formation of large quantities of aflatoxins. This could be as a result of the quality of the oil content of the Shea nut, although it supports good colonization and growth of *Aspergillus* species. The reasons for low aflatoxin formation are yet to be investigated. Also, the water activity in the study does not significantly influence the amount of aflatoxin formation in Shea.

1.6. CONCLUSION AND RECOMMENDATIONS

Time of picking of the nut is critical in the quality of butter that is obtained. Farmers often gather the nuts to have a greater quantity before processing and in the process fruit decomposition by microorganisms eventually is common. Fruits should be should be picked up and processed immediately.

From this study the drying process/stage is a critical stage that should be given priority in the processing of premium quality Shea-kernel/butter, and this can be achieved by the use of electric dryers or fuel-powered driers, which will facilitate quick rapid drying of the nuts/kernels and prevent contaminants from proliferating.

Sheanut farmers often roast the crushed kernels over naked flame to enhance butter production. This smoke introduces poly-cyclic aromatic hydrocarbons on the butter some of which are potential carcinogens. Use of roasters will prevent direct contact of the butter with smoke thereby enhancing the better butter quality.

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