THE IMPACT OF ASSOCIATED FUNGI ON POST-HARVEST DETERIORATION OF SHEA KERNELS IN NIGERIA

[1,2*]Udoh M.E., [2]Jonathan S.G., [1]Esiegbuya D.O., [1]Ekhorutomwen O. E.., [3]Owoh B.N. and [1]Omoregie K.O.

[1]Plant Pathology Division, Nigerian Institute for Oil Palm Research (NIFOR), PMB 1030, Benin City, Edo State.

[2]Department of Botany, Faculty of Science, University of Ibadan, Ibadan, Nigeria.

[3]Physiology and Tissue Culture Division, Nigerian Institute for Oil Palm Research (NIFOR), PMB 1030, Benin City, Edo State.

*Corresponding author's email address: sleekymay@gmail.com; Phone No.: +2347039551379

SUMMARY

Shea kernels constitute a major source of income and nutritional components for a vast majority of the population in many African countries including Nigeria, particularly the rural areas and Nigeria has been reported to be the largest producer of shea but the recurring problem of mouldiness of Shea nuts/kernels and high free fatty acid 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 unfortunately is a major threat to food security. Three Shea- producing states in Nigeria (Kwara, Oyo and Niger) were investigated for fungi impact on shea kernels and samples were analysed for fungi contamination and its identification. The study examined the types of storage houses and materials used for shea products (kernels and butter) storage and the associated pathogens of *Vitellaria paradoxa* (Gaertn.). Results from this study revealed the presence of fungi-mould on kernels which was most prevalent. Eight fungal species from the genera *Aspergillus, Trichoderma* and *Penicillium* were encountered from the samples collected in the selected ecological zones covered in the survey. Samples from Kwara state had the highest frequency rate of microbial contamination at 95% followed by Niger State which had a moderate frequency rate of microbial contamination at 50% and Oyo State having the least frequency rate of microbial infection at 25%. The characteristic rapid deterioration of shea products coupled with the total absence of effective storage management system, have already put the Shea industry in Nigeria in a serious menace. It is expedient to urgently develop a long-term storage approach for shea and its products in order to generally sustain shea industry worldwide.

Keywords: Shea kernels, storage, quality, temperature, fungi mould, deterioration.

Vitellaria paradoxa (Shea butter tree) has been recognized in the Guinea and Sudan savanna zones of Nigeria to be one of the most economic indigenous crop grown between latitudes 70-120 N (Warra, 2009). Shea butter and its raw materials are widely used to treat skin problems such as burns, dryness, ulcers, sunburns and dermatitis (Bonkougou, 1987; Fakayode *et al*., 2013). The tree naturally grows in the wild and its abundance in Nigeria exists and thrives almost exclusively in the North. Nigeria is the largest producer of Shea nuts (425,000MT) but contributes relatively little (45,000MT) to world exports (Olife *et al*., 2013). Shea butter tree plays a socio-economic vital role in poverty alleviation and food security among Nigerians most especially in terms of employment and income generation to a significant proportion of rural population especially women who are, directly involved in Shea nut collection and butter extraction (Matanmi *et al*., 2011).The plant is currently exploited for butter, charcoal, firewood, mortar and pestle. Its fatty matter has been explored over the years in Africa for different purposes ranging from food and soap processing to healthcare and other medicinal uses (Coulibaly *et al*., 2009). Shea kernels storage and handling have a great effect on the quality of Shea butter extracted from the kernels. Storage can be a major problem in Nigeria because majority of the Shea growing regions are located in the tropics, where the combination of high temperature and high relative humidity support various fungal growth which cause rapid deterioration of seed quality. Storage conditions are key constraints for quality assurance of the shea butter. These conditions could impact the quality of the products (butter and oil) and reduce their access to international market. The aim of this study is to examine the storage system practices and handling of Shea kernels in the shea cottage industry and its effect on the quality of the kernels and its by-product (butter).

MATERIALS AND METHOD

Study area

A survey was conducted in 3 Shea- producing states in Nigeria (Kwara, Oyo and Niger) between the production seasons (June-August). These areas cover most of the agro-ecological zones for Shea production in Nigeria. It also comprises Shea villages, Shea farmlands, few storehouses and Shea markets. The sites in each state were selected with the aid of extension officers of the

Agricultural Development Programme (ADP) in the states visited. Information about Shea processing and storage was collected from farmers and processors in the locations visited. The information gathered from farmers and processors aided in the identification of challenges in storage of Shea kernels.

Experimental site

The present study was conducted in the field and in the laboratory. The field study was carried out in the Rainforest, Derived Savannah and Guinea Savannah regions of Nigeria where there is extremely high densities of Shea tree distribution and processing activities. The laboratory analysis was carried out in the Plant Pathology/Mycology laboratory of the Department of Botany, University of Ibadan, Plant Pathology laboratory of the Nigerian Institute for Oil Palm Research (NIFOR), Benin City and Biotechnology laboratory of the International Institute for Tropical Agriculture (IITA), Ibadan. The geographical coordinates of each location were taken using a Geographical Positioning System (GPS) device and the respective sampled regions are illustrated in (Fig. 1).

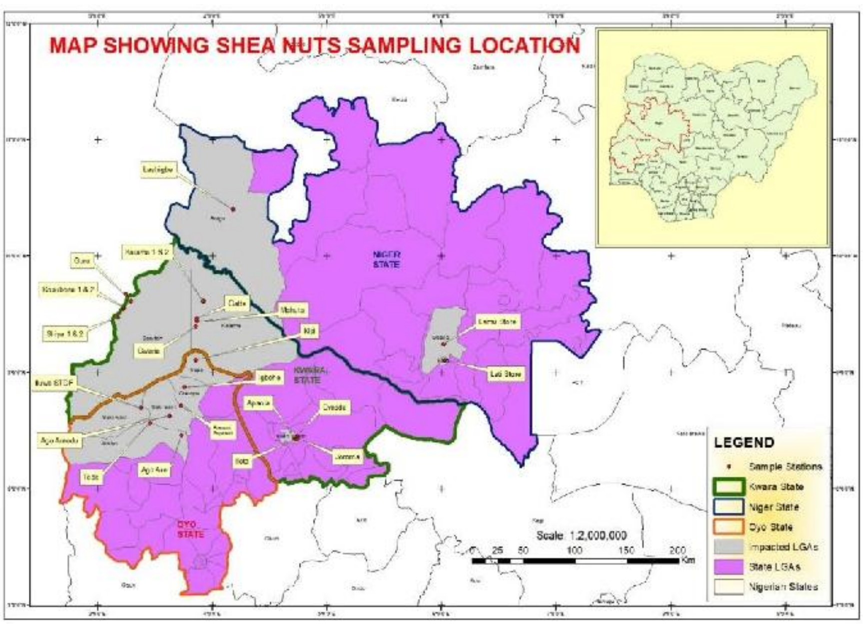

Figure 1: Niger, Kwara and Oyo States of Nigeria, indicating locations of shea kernels collection.

Types of storage system practices of shea kernels and nuts by shea farmers and processors in the study areas surveyed.

The storage system practices in the three states used for this study were evaluated to determine the mycoflora associated with the stored shea kernels. The methods of storage for the harvested shea nuts and kernels varied from one agro ecological zone to another. Most Shea farmers and processors stored their shea kernels after drying in jute bags, some stored the nut in agro sac bags, others in cement bags, rice bags, waterproof bags, on a thatch roof (AJA), basins and on the ground respectively after which the nuts and kernels are kept in an open air ventilated and non-ventilated room.

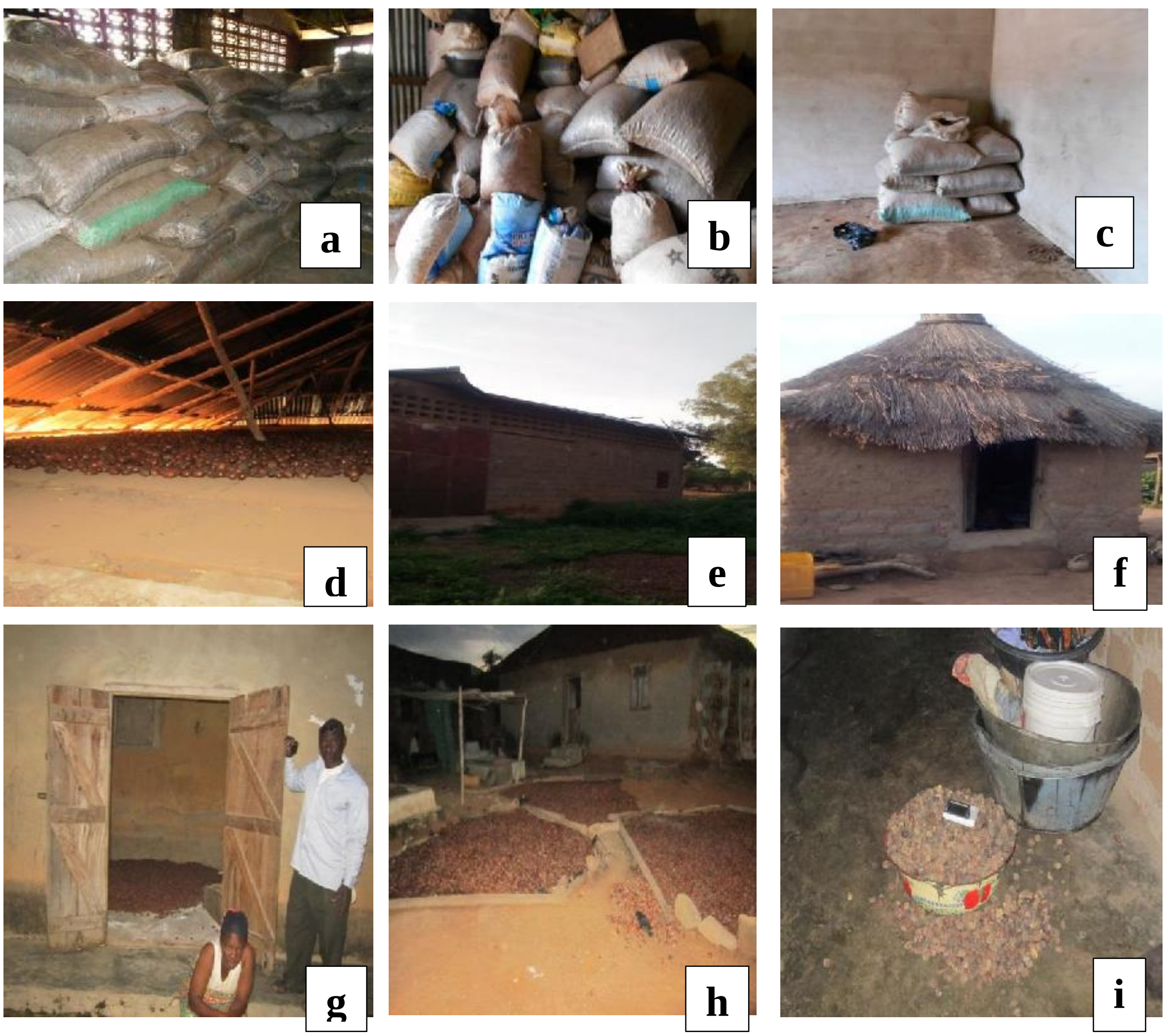

Figure 2 : Storage houses and materials for Shea kernels/nuts storage from the sampled locations. **(a.)** Dried Shea kernels stored in a polyethylene sac bags in an air ventilated warehouse (**b.)** poor storage conditions of dried shea kernels stored in agro chemical sac bags **(c.)** a private warehouse maintained by a wholesaler where dried Shea kernels are stored for distribution to marketers and processors **(d.)** unprocessed shea nuts stored on a roof top building "**AJA**" **(e.)** a public warehouse owned by a semi government body for traders/retailers usage **(f.)** a locally constructed warehouse for shea kernel storage **(g, h)** drying of depulped shea nuts on a cemented and bare ground **(i.)** poor storage condition of dried shea kernels in basin.

Sample collection and preparation

Healthy and diseased samples of shea kernels were randomly collected from different domestic districts in each of the States (i.e. Oyo, Niger and Kwara). 5 kg sample of dried kernels (DK) n = 10 was collected from each of the following locations: Igboho in Oorelope LGA, Kisi in Irepo LGA, Iluwa standard and trade development facility project site in Saki West LGA, Ago-Are and Tede in Atisbo LGA, Ago Amodu, Sepeteri and Araromi-Sepeteri in Saki East LGA (Oyo State); Tadafu, Lati store and St. John storehouse in Bida LGA, Batako, Ladigi and Lemu storehouses in Gbako LGA, Wawa-Karamu, Wawa- Lagabi and Leshigbe storehouses in Borgu LGA (Niger State); Gure, Kosubosu and Shiya in Baruten LGA, Apaola, Ilota, Omode and Joroma in Ilorin South LGA, Gatte, Gwaria, Mahuta and Kaiama in Kaiama LGA (Kwara State) and Tasso in Borgou LGA in Republic of Benin. Shea kernels were collected aseptically packaged in sterile polyethylene bags, labelled and transported to the laboratory for disease diagnosis and further analysis. The temperature and relative humidity at the time of sampling was collected using a digital thermo hygrometer. One gram of each of the diseased sample was weighed out and macerated using sterile pestle and mortar. The macerated sample was aseptically transferred into 10 ml of sterile distilled water. It was then placed on a rotary shaker for 10 mins to dislodge the pathogen. Appropriate fivefold serial dilution was thereafter carried out and 1 ml of the stock solution was aseptically transferred into 9 ml sterile distilled water to make the dilution factor of

10^{-1}. The procedure was repeated for 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} respectively. The sub-samples were used for isolation, colony forming unit (CFU/g) counts, determination of environmental factors for storage and aflatoxin analysis.

Sorting

Diseased shea kernels were sorted out, aseptically packaged and labelled differently from the healthy shea samples for each location prior to the laboratory analysis.

Isolation of post-harvest spoilage fungi

The pour plate method of Barnett and Hunter (1987) was employed for the isolation of fungi. One ml of the serially-diluted sample (10^{-3} and 10^{-5}) was aseptically dispensed into sterile Petri plates containing potato dextrose agar (PDA). Incubation was carried out at 28ºC for 5 days. The colonies that developed were counted, recorded and sub-cultured repeatedly on to fresh PDA plates to obtain pure cultures. The pure cultures were later stored on PDA slants for characterization and identification. Identification of pure isolates was done at the International Institute for Tropical Agriculture, IITA using the DNA Extraction technique. For the DNA isolation and molecular identification of the isolates, fungal cultures were grown and maintained in Czapek Dox broth ($NaNO_3$ 2g/l, KH_2PO_4 1g/l, $MgSO_4.7H_2O$ 0.5g/l, KCl 0.5g/l, $FeSO_4.7H_2O$ 0.01g/l, sucrose 30g/l, distilled water 1000 ml) with a pH adjusted at 6.5 by 1 N NaOH/ 1 N HCl and incubated at 28 ± 1º C for 7 days on continuous shake of 115 rpm. Mycelia mass were filtered using Whatman filter paper No. 1 and DNA was extracted using DNA Extraction buffer (DEB). Genomic DNA was extracted from mycelium following the Dellaporta method (1983). 100g of fungal mycelia were transferred into sterile mortar and 1 ml of DNA Extraction buffer containing proteinase K (0.05mg/ml) was added and mycelia were macerated with a sterile pestle.

Extract of finely macerated fungal mycelia was transferred into 1.5 ml eppendorf tube. 50 µl of 20% Sodium Dodecyl Sulphate (SDS) was added to fungal mycelia extract and incubated in a water bath at 65ºC for 30minutes and allowed to cool to room temperature. 100µl of 7.5 M

Potassium acetate was added to suspension and mixed briefly. The resulting suspension was centrifuged at 13000rpm for 10minutes. Supernatant was transferred into new autoclaved tubes. To the supernatant, 2/3 volumes of cold isopropanol /isopropyl alcohol was added, mixed carefully and incubated at -20ºC for 1hour. Supernatant was centrifuged at 13000rpm for 10minutes and discarded. DNA was washed carefully with 500µl of 70% ethanol and centrifuged for 5minutes at 13000rpm. DNA pellets was dried at 37ºC for 10-15 minutes and re-suspended in 50µl of Tris-EDTA (TE, 10mM Tris-HCl, pH 8.0, 1mM EDTA) buffer.

Evaluation of storage condition of shea kernels

(i) Measurement of temperature and relative humidity

The temperature and relative humidity from the various location of sample collection at the time of collection were recorded using the Digital thermohygrometer with internal temperature range of -25º C - +85º C and relative humidity range of 0 – 100%. The instrument was placed within and outside the stored environment where shea kernels samples were collected and the temperature and relative humidity of each storage environment were recorded.

(ii) Determination of shea kernel moisture content

The shea kernel moisture content was measured using digital weigh balance before storage. Exactly, 10g of each shea kernel sample was weighed and placed in a pre- weighed envelope and dried to a constant weight at 105º C in a drying oven. The moisture content of each sample was determined using the AOAC 1990 Official method of analysis;

$$\% \text{ Moisture content} = \frac{W_1 - W_2}{W_1 - W_0} \times 100$$

Where W_0 = Weight of empty crucible

W_1 = Weight of crucible + sample

W_2 = Weight of crucible + oven dried sample

Data analysis

Descriptive statistics such as mean, frequency and percentage were performed on different variables.

RESULTS

Visual examination of shea nuts/kernels

The samples collected from the different locations were observed for its colour, texture and smell. There was a characteristic difference in colour among the samples obtained from industrial storage materials and non-industrial storage materials. The colour of infected samples in general was greenish yellow while some were brownish and sooty black in texture. Samples from Kwara in particular were greenish yellow, while those from Niger were mouldy black and that from Oyo were mixture of mouldy colours (Plate 4).

Plate 1: A- Infected Shea kernels from Niger **B-** Infected Shea kernels from Kwara
C- Infected Shea kernels from Oyo **D-** Healthy Shea kernels

Evaluation of environmental parameters on shea kernels storage

The environmental parameters such as temperature, relative humidity of storage house was measured (Table 1) and the percentage moisture content on shea kernels obtained from each sampled locations was also determined using the official method of analysis.

Table1: Environmental and physical parameters of sampled locations of storage shea kernels

State	Location	LGA(s)	Temperature (℃)	R.H (%)	Percentage Moisture content (%)
Niger	LS 1	Bida	30.5	65	11.83
	LS 2	”	32.0	63	12.23
	LS 3	”	29.5	68	5.44
	SJ 1	”	34.2	65	9.50
	SJ 2	”	33.1	63	8.40
	T1	”	28.5	74	20.68
	T2	”	27.0	72	18.45
	LE	Borgu	25.2	75	9.24
	WK	”	30.1	62	14.74
	WL	”	31.2	60	18.03
	B1	Gbako	28.5	75	11.70
	B2	”	25.5	82	18.29
	L1	”	27.2	79	9.14
	L2	”	25.0	75	7.57
	LA	”	26.5	83	11.65
Oyo	AA	Atisbo	28.9	62	26.18
	T	”	31.2	61	16.67
	K	Irepo	32.4	63	12.24
	IG	Oorelope	27.9	65	19.55
	IL	Saki West	27.1	74	11.82
	AAm	Saki East	34.5	55	46.05
	AS	”	31.8	60	38.81
	S	”	31.9	61	44.11
Kwara	G	Baruten	33.1	54	14.07
	K1	”	26.1	76	36.20
	K2	”	25.9	79	10.63
	S1	”	25.7	82	18.43
	S2	”	27.0	77	9.52
	AO	Ilorin South	27.4	78	12.45
	I1	”	26.6	82	10.13
	I2	”	26.7	81	12.81
	J	”	26.7	82	11.74
	O	”	26.8	82	4.92
	Ga	Kaiama	30.3	62	11.21
	Gw	”	33.4	49	12.38
	M	”	32.2	61	13.05
	K1	”	36.1	44	14.93
	K2	”	37.5	41	6.25

Keynotes: Niger locations- LS (Lati store), SJ (Saint John), T (Tadafu), LE (Leshigbe), WK (Wawa, kara), WL (Wawa, Lagabi), B (Batako), L (Lemu), LA (Ladigi). Oyo locations- AA (Ago-Are), T (Tede), K (Kisi), IG (Igboho), IL (Iluwa), AAm (Ago-Amodu), AS (Araromi Sepeteri), S (Sepeteri). Kwara locations- G (Gure), K (Kosubosu), S (Shiya), AO (Apa-ola), I (Ilota), J (Joroma), O (Owode), Ga (Gatte), Gw (Gwaria), M (Mahuta) and K (Kaiama).

Table 2: Colony count of fungal isolates obtained from deteriorated shea kernels from Niger, Oyo and Kwara State.

State	Location	LGA(s)	Mean colony count (CFU)	Fungal Isolates
Niger	LS 1	Bida	$21x10^4$	*Aspergillus flavus*
	LS 2	”	$32x10^4$	*A. niger* *A. fumigatus*
	LS 3	”	$4x10^4$	*Aspergillus niger*
	SJ 1	”	$13x10^4$	*A. niger*
	SJ 2	”	$33x10^4$	*A. flavus*
	T1	”	$12x10^4$	*A. niger* *A. fumigatus*
	T2	”	$27x10^4$	*A. niger*
	LE	Borgu	$5x10^4$	*A. niger* *A. fumigatus* *Trichoderma asperellum*
	WK	”	TNC (□100)	*A. niger*
	WL	”	TNC (□100)	*A. niger* *A. flavus*
	B1	Gbako	$14x10^4$	*A. niger* *A. flavus*
	B2	”	$25x10^4$	*A. fumigatus*
	L1	”	$27x10^4$	*A. niger* *Aspergillus flavus*
	L2	”	$2x10^4$	*A. niger*
	LA	”	TNC (□100)	*A. niger* *A. flavus*
Oyo	AA	Atisbo	TNC(□100)	*Aspergillus niger*
	T	”	$15x10^4$	*Penicillum* sp
	K	Irepo	$20x10^4$	*Aspergillus niger* *Penicillum* sp
	IG	Oorelope	TNC(□100)	*Aspergillus niger* *Aspergillus fumigatus*
	IL	Saki West	$12x10^4$	*Aspergillus niger* *Aspergillus flavus*
	AAm	Saki East	$31x10^4$	*Aspergillus sp* *Penicillum sp*
	AS	”	$31x10^4$	*Aspergillus niger* *Trichoderma harzanium*
	S	”	$4x10^4$	*A. niger* *A. flavus* *Penicillum sp*
Kwara	G	Baruten	$46x10^4$	*Trichoderma asperellum* *A. niger*
	K1	”	$23x10^4$	*A. niger*
	K2	”	$58x10^4$	*A. niger* *A. flavus*
	S1	”	$57x10^4$	*A. tamari*
	S2	”	$184x10^4$	*A. niger*

AO	Ilorin South	TNC(□100)	*Aspergillus niger*
I1	”	$132x10^4$	*Aspergillus sp*
I2	”	$184x10^4$	*Aspergillus flavus*
J	”	$48x10^4$	*Aspergillus fumigatus*
O	”	$168x10^4$	*Aspergillus fumigatus* *Penicillum* sp
G	Kaiama	$156x10^4$	*A. niger*
Gw	”	$188x10^4$	*A. niger* *T. harzanium*
M	”	$96x10^4$	*Aspergillus flavus*
K1	”	$23x10^4$	*A. niger*
K2	”	$58x10^4$	*A. niger* *A. flavus*

Keynotes: TNC – Too numerous to count

Table 1 shows the environmental and physical parameters of stored shea kernels obtained from the sampled locations of study. Samples from Niger State had percentage moisture content ranging from 5.44% - 20.68% at a temperature and R.H (29.5℃; 68% - 28.5℃; 74%), Oyo State ranging from 11.82% - 46.05% at a temperature and R.H (27.1℃; 74% - 34.5℃; 55%) and Kwara State ranging from 4.92% - 36.20% at a temperature and R.H (26.8℃; 82% - 26.1℃; 76%). Environmental differences were highly observed from storage environments in Oyo State compared to Niger and Kwara State respectively. Samples of Shea kernels obtained from Borgu and Gbako LGA at a temperature range of 26.5℃ - 31.2℃ had a significant microbial growth yield of *A. niger and A*. flavus (□ 100), while samples from Bida, Borgu and Gbako (Niger State) having the least moisture content of 5.44%, 7.57% and 9.24% yielded a less significant microbial growth of *A. niger, A. flavus* and *T. asperellum* ($2\text{-}5x10^4$). Shea kernels samples at moisture content 19.55% and 26.18% yielded a significant growth of *Aspergillus* spp. (□100) at a temperature range of 26.5℃ - 27.9℃ and R.H of 62% and 65% respectively from Atisbo and Oorelope LGAs in Oyo State while from Saki East LGA (same Oyo State) had a striking significant difference in microbial growth of *Aspergillus* spp. and *Penicillum* spp. ($4x10^4$) with a high percentage moisture content of 44.11%. Results of study from Kwara State (Ilorin South and Kaiama LGAs) revealed a high level of microbial growth mainly from *Aspergillus* spp. (□ 100) within a temperature range of

26.6℃-33.4℃ and R.H range of 49% - 82% while there was little or less significant growth yield as observed in Niger and Oyo State.

Table 2 shows the fungi identified and mean colony count of fungal isolates obtained from deteriorated shea kernels from Niger, Oyo and Kwara State. The fungal isolates from the shea kernels were *A. niger*, *A. flavus*, *T. harzianum*, *A. fumigatus*, *Aspergillus* section *Nigri*, *T. asperellum*. The most predominant fungal isolates with a high level of microbial contamination of the samples are species of the genera *Aspergillus* with *A. niger* emerging as the most occurring species obtained in all the samples.

Table 3: Percentage frequency occurrence of fungal isolates associated with deteriorated shea kernels

Fungus	Total number of isolation	Frequency (%)
Aspergillus flavus	12	21
Aspergillus niger	27	47
Aspergillus fumigates	7	12
Aspergillus sp	2	3
Aspergillus tamari	1	2
Trichoderma asperellum	2	3
Penicillum sp	5	9
Trichoderma harzanium	2	3

Table 3 presents the percentage frequency occurrence of fungal isolates associated with deteriorated shea kernels. *Aspergillus niger* (47%) had the highest frequency of occurrence from the total number of fungal isolates obtained in the sampled locations, followed by *A. flavus* (21%) and *A. fumigatus* (12%). The least frequency of occurrence was observed in the following fungal isolates; *Penicillum* spp. (9%), *T. harzanium* and *T. asperellum* (3%), *Aspergillus* spp. (3%) and *A. tamari* (2%). From this study, it was observed that *A. niger* is the most predominant fungal isolate associated with deterioration of shea kernels while *A. tamari* is less predominant even though the study reveals its association in the deterioration process.

DISCUSSION

The major physicochemical and biochemical changes caused in shea kernels by the growth of eight storage fungi showed that the fungi had caused a hydrolytic type of deterioration in the shea kernels. Associated with this hydrolysis was a discoloration of the kernels as well as the butter. These results agree with the observations of (Iqbal *et al*., 2014) who have investigated the effect of individual fungal species on other oil seeds. The *Aspergillus* spp. associated with the contamination of storage shea kernels was similar to those previously reported by Esiegbuya *et al*. (2014) which cause kernels and butter discolouration and deterioration thereby resulting in colour change of the kernels and butter. The authors stated that *Aspergillus* spp. (*A. niger, A. persii, A. flavus and A. fumigatus*) cause postharvest deterioration of the shea nuts and kernels. It has been discovered in earlier researches that *A. aggregate, A. fumigatus, A. oryzae and A. niger* have the ability to reduce the physical and chemical quality of the shea butter. In this study the *Aspergillus* spp. isolated were mainly *Aspergillus* Section *Nigri* and *Aspergillus,* section *Flavi*. Species of the genus *Aspergillus* Section *Nigri* are widely distributed and have a capacity to grow in variety of substrates and produce metabolites such as citric and gluconic acids, as well as hydrolytic enzymes (Abarca *et al*., 2004; Valero *et al*. 2007). They are also known to include the uniseriate and biseriate species. (Samson *et al*., 2004).

The genus *Aspergillus,* section *Flavi* species on the other hand are important because they cause serious damage to stored food commodities and also have the ability to produce mycotoxins (Baquião *et al*., 2013). They include the *A. flavus, A. parasiticus, A. nomius, A. oryzae, A. sojae* and *A. tamari*. Though these species share numerous common features, but differ in their attribute to produce aflatoxins; thus dividing them into the nonaflatoxigenic and aflatoxigenic groups (Rodrigues *et al*., 2011).

The significant increases in moisture content of kernels caused by the fungi would suggest that these fungi were carrying out a lot of metabolic activities in the kernels which led to their deterioration; a high amount of moisture will normally support the fast growth of existing fungi and encourage the proliferation of new ones. Increases in moisture content of infected oil seeds caused by *A. niger* have been reported as a major contaminant of peanuts, maize, and grains (Cheeseborough, 2005) which are the most popular staple foods in Africa (Thomas *et al*., 2012). *Aspergillus niger* were mostly found in the kernels with greatest increase in moisture content in this investigation.

CONCLUSION

The characteristic rapid deterioration of shea products coupled with the total absence of effective storage management system, put the Shea industry in Nigeria in a serious menace. It is expedient to urgently develop a long-term storage approach to salvage shea industry world wide. Storage practices of shea nuts and kernels study is a necessary step for the characterization of fungi associated with deterioration of shea and its products. The study will aid the evaluation of the fungi diversity of shea deterioration in the Guinea Savannah provinces of Nigeria. The entire value of Shea nut industry depends on the individual value of the diverse chain of production.

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