Journal of
Microbial & Biochemical Technology

Jonathan Segun et al., J Microb Biochem Technol 2017, 9:5
DOI: 10.4172/1948-5948.1000372

Research Article Open Access

Food Values, Spoilage Moulds and Aflatoxin Detection in 'Attiéké' (A Cassava Fermented Product)

Jonathan Segun G*, Bello Tunde S and Asemoloye Michael D

Department of Botany, Food and Environmental Mycology/Biotechnology Unit, University of Ibadan, Ibadan, Oyo State, Nigeria

Abstract

Derived foods from root and tuber crops, Attiéké for example, are often consumed by African populace. Attiéké is processed from Cassava (*Manihot esculenta* Crantz). Based on different methods adopted for its processing and storage, we present the food values, bio-deteriorating/spoilage fungi and aflatoxin contents of Attiéké samples, collected from different locations in Nigeria and Ivory Coast. Aflatoxin contents were detected using high Performance Liquid Chromatography (HPLC). Result obtained shows that the most frequent fungal contaminants in the samples are *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, *Mucor hiemalis* and *Penicillium chrysogenum*. Records on the aflatoxin contents shows that the food samples contain AFB_1 (1.03-6.72 µg kg^{-1}), AFB_2 (2.46-2.56 µg kg^{-1}) and AFG_1 (1.43-9.57 µg kg^{-1}) range. It is also observed that the samples contain appreciable amount of Crude Protein (0.48-0.73%) and Moisture Content (45.89-49.96%) ranges with storage time, percentage Crude Fibre (CF) range from 1.08-1.12%, 0.14-0.18% Crude Fat (EE) and 0.45-0.49% Percentage Ash.

Keywords: Attiéké; Food values; Moulds; Aflatoxins;Tolerance limit; Health threat

Introduction

Root and tuber crops are of immense importance to the feeding habit of African populace [1]. Cassava (*Manihot esculenta* Crantz) is consumed in various forms including Attiéké. Attiéké is becoming a daily intake for people in West and Central Africa that has a bed rock in it as an energy source [2]. It is a starchy-couscous dish derived from fermented Cassava dough processing and most importantly produced by Ivorian particularly the coastal population of the country [3]. Its appreciation is going beyond boundaries as a staple food due to black African-diaspora immigration [4]. In Cote d'Ivoire, Attiéké plays a large part in household sustenance with regards to its combat against hunger and also its nutritional supplements [5]. Attiéké is acknowledged to be representing 5% food expenditure and 20.5% Calories diet daily intake for consumers and its production is approximately estimated around 18965 to 40000 tons and consumption per inhabitants lies between 28 and 30 kg annually [5-8]. Its production still follows conventional procedure and not on modern procedure [9-11].

The preparation of attieke from cassava varies and numerous across different communities, though, the most complex aim is eliminating its bitterness and toxicity [12]. To avoid this, fermentation is carried out in numerous traditional transformation technologies of Cassava roots [13]. Its production proceed in the unit operations including peeling, grating, fermenting, pressing, crumbling, sieving, semolining, drying, air winding and cooking [14,15]. These units of operations proceeds traditionally, but unhygienic handling may lead to faster fungal deterioration and a resultant mycotoxicity.

Fungi which were ignorantly believed to be an anaesthetic organism growing on food had been lime lighted when the famous Turkey X diseases claimed lives of approximately 10,000 Turkish and lesser domestic birds in Great Britain [16]. This became informative that spoilage fungi could produce toxins named Aflatoxin: "A" obtained from *Aspergillus* and "fla" from *flavus,* where the name of the organism *Aspergillus flavus* was derived. Conditions that predispose food to these organisms include; hot and humid climate, damage by insects that decrease host's immunity, moisture content of 16% and above [17].

This research was embarked upon to investigate food values, spoilage moulds and aflatoxin detection in Attiéké from Nigeria and Ivory Coast.

Materials and Methods

Collection of samples

Samples of prepared ready for cooking 'Attiéké' (i.e., processed cassava dough) were randomly selected from two major table top food sellers in three (3) locations where 'Attiéké' has its best cook and very demanding consumers as shown below:

1. a) Ejigbo, Beulah Church (6°33'8"N, 3°18'26"E)

   b) Ejigbo (Ore meji), (6°30'8"N, 3°18'20"E)
2. a) Iwo, BHS (7°47'00N, 4°12'00"E)

   b) Iwo, Odo, ori market (7°46'00N, 4°12'00"E)
3. a) Adjame Bromokoute 1 (5.36°N, 4.02°W)

   b) Adjame Bromokote 2 (5.36°N, 4.02°W)

These locations are tropics, according to climate data; they have an annual rainfall of 1247 mm, 1264 mm and 1781 mm, respectively which last from April to October in Ejigbo and Iwo and from January to June in Adjame.

Variations due to different days of cooking were worked out such that they were all steamed at the same time. A total of six (6) samples were collected (2) samples from each location. The cooked samples

***Corresponding author:** Jonathan Segun G, Department of Botany, Food and Environmental Mycology/Biotechnology Unit, University of Ibadan, Ibadan, Oyo State, Nigeria, Tel: +2348164746758; E-mail: asemoloyemike@gmail.com

Received September 10, 2017; **Accepted** October 25, 2017; **Published** October 31, 2017

Citation: Jonathan Segun G, Bello Tunde S and Asemoloye Michael D (2017) Food Values, Spoilage Moulds and Aflatoxin Detection in 'Attiéké' (A Cassava Fermented Product). J Microb Biochem Technol 9:244-248. doi: 10.4172/1948-5948.1000372

were placed in clean sterile polythene bags, securely tied, labeled and transported to the laboratory.

Research treatments and design

Appreciable gram of each sample was taken and divided into three (3); one-third was stored in the freezer at subzero degree for aflatoxin analysis, the second fraction was stored at room temperature for nutrient analysis and the other was used immediately for isolation of fungi. The experiment was laid in Completely Randomized Design with three replicates.

Isolation and characterization of fungi biota

The isolation of fungi was carried out according to procedure described by Jimoh and Kolapo [18]. All samples collected were conditioned in a sterile package. About 2 g of each of the samples taken at random were aseptically placed in three replicates of Petridishes containing Potato Dextrose Agar and Lactic acid (10.53 gL^{-1}). The dishes were incubated at 27 ± 2°C for 3-6 days. Fungal cultures obtained were subsequently sub-cultured for purification. Upon fungi maturation, they were characterized based on cultural and morphological features such as colony diameter, colony color on agar, front and reverse and colony texture. Slide culture was then prepared and incubation in moist chambers at 26 ± 2°C for 4 days before observation under a light microscope (labomed, model CxL). Mycological keys and manuals were used for macro and microscopic features that are commonly used identification of fungi, which were conidiophores, conidial shape, phialides and metulae, presence and shape of vesicles [19].

Aflatoxin detection

The modified method of using HPLC chromatography was followed in determining aflatoxin concentrations of Aflatoxin B1 (AFB_1), Aflatoxin B2 (AFB_2) and Aflatoxin G1 (AFG_1) [20]. The samples were injected into the HPLC column heated to 40°C using mainly reversed-phased columns, with mobile phases composed of water: methanol solution (60:40, v/v). To 1 L of mobile phase were added 119 mg of Potassium bromide and 350Bl of 4 M nitric acid (required for post/column electrochemical derivatisation with Kobra Cell, ROBiopharm Rhone). This method is used after an extraction with acetonitrile and water, reaching limits of qualification between 0.012 and 0.073 µg kg^{-1} was used. The coupling of HPLC to mass spectrometry was also used for the detection technique at the excitation wavelength of 362 nm and the emission wavelength was 425 nm. The ionization sources employed was based on atmospheric pressure [21,22]. The sample was calculated by analyzing triplicates of six samples. The method showed recoveries between 95% and 114% with a LOQ of 1.5 $ngmL^{-1}$.

Crude protein determination

The crude protein in the samples was determined by the routine semi-micro Kjeldahl, procedure. 0.5 g of each finely ground dried sample was weighed carefully into the Kjeldahl digestion tubes, 1 tablet of selenium catalyst and 10 ml of conc.H_2SO_4 was added into the digestion preset at 500°C. The digestion was left for 4 h in a fume cupboard. The tube was placed in a distilling unit and 5 ml of 40% NaOH was added to it. The mixture was steam distilled for 2 min into a 50 ml flask containing 10 ml of 2% Boric acid, mixed with indicator solution which was then titrated against 0.01 N HCl until a wine color was obtained. The quantification was done using AOAC procedure [23].

Crude fat determination

1 g of each dried sample was weighed into fat free extraction thimble and plug lightly with cotton wool. A soxhlet flask was then filled to ¾ of its volume with petroleum ether and the ether was left on heater to siphon until it was short of siphoning. Ether content of the extractor was drained into the ether stock bottle. The thimble containing sample was then removed and dried on a clock glass on the bench top. The extractor, flask and condenser were replaced and the distillation continued until the flask was practically dry. The flask containing the fat was detached; its exterior cleared and dried to a constant weight in the oven, and the crude fat was determined [23].

Dry matter and moisture determination

2 g of the sample were weighed into a previously weighed crucible (Wo). The crucible plus sample (W_1) taken was then transferred into the oven set at 100°C to dry to a constant weight for 2 h. At the end, the crucible plus sample was removed from the oven and transferred to desiccators, cooled for ten minutes and weighed (W_3) [23].

$$\%\ \text{Dry Matter}\ (\text{DM}) = \frac{W_3 - Wo}{W_1 - Wo} \times 100$$

% Moisture=100 – % DM.

Ash determination

2 g of the sample was weighed into a porcelain crucible. This was transferred into the muffle furnace set at 550°C and left for about 4 h. About this time it had turned to white ash. The crucible and its content were cooled to about 100°C in air, then room temperature in a desiccator and weighed [23].

$$\text{Ash content} = \frac{\text{weight of ash}}{\text{Original weight of sample}} \times 100$$

Fibre determination

2 g of the sample was weighed accurately into the fibre flask and 100 ml of 0.255 N H_2SO_4 was added. The mixture was heated under reflux for 1 h with the heating mantle. The hot mixture was filtered through a fibre sieve cloth. The residue was returned to the fibre flask to which 100 ml of (0.313 N NaOH) was added and heated under reflux for another 1 h. The mixture was filtered through a fibre sieve cloth and 10 ml of acetone added. The residue was washed with about 50 ml hot water on the sieve cloth before it was finally transferred into the crucible. The crucible and the residue were oven-dried at 105°C overnight to drive off moisture. The oven-dried crucible containing the residue was cooled in a desiccator and weighed to obtain the weight W_1. The crucible with weight W_1 was transferred to the muffle furnace for ashing at 550°C for 4 h. The crucible containing white or grey ash was cooled in the desiccator and weight to obtain W_2. The difference $W_1 - W_2$ gives the weight of fibre [23].

$$\%\ \text{Fibre} = \frac{W_1 - W_2}{\text{Weight of sample}} \times 100$$

Statistical Analysis

Data were subjected to Statistical Analysis of Variance (ANOVA) at 95% and 99% probability levels using SAS 9.3 statistical package and means were separated using Duncan Multiple Range Test.

Results

The result in Table 1 shows the Mean Nutritional composition in wet Attiéké samples collected from different location under different storage periods. The samples were highly significant ($P<0.01$) for crude protein and moisture contents, but significant ($P<0.05$) for Crude fibre, Crude Fat and ash contents. It was observed that that all the samples were significantly different from each other for crude protein. The

crude protein obtained from samples from Ivory Coast stored for three days was significantly higher (P<0.05) than other samples while the least mean value was obtained from sample collected from Nigeria with storage period of a day (Table 1).

The result also shows that the Crude fibre of sample collected from Ivory Coast stored for one day is significantly higher but not different from Ivory Coast samples stored for two days. Also, sample collected from Nigeria stored for one day and three days were non significantly different from each other, but different from sample collected from Nigeria stored for two days and sample collected from Ivory Coast stored for three days were not significantly different from each other.

The Crude Fat of samples from Nigeria stored for three days were not significantly (P>0.05) different from Ivory Coast samples stored for three days. The highest mean value of Wet Attiéké Crude Fat was recorded from Nigeria stored for one day. The ash content of samples collected from Ivory Coast stored for two days and three days were not significantly different from each other while the least ash content of Wet Attiéké was obtained from Nigeria sample stored for two days.

Highest moisture content was observed from samples collected in Ivory Coast stored for three days while the least moisture content of Attiéké was obtained from Nigeria sample stored for two days.

Nigeria stored for one day; A18, Nigeria stored for two days; A19, Nigeria stored for three days; A20, Ivory Coast stored for one day; B18, Ivory Coast stored for two day; B19, Ivory Coast stored for three day;B20.

Results obtained in this study showed that the spoilage fungi found associated with Attiéké include; *Penicillium chrysogenum, Aspergillus niger, Candida albicans, Aspergillus flavus* and *Mucor hiemalis* as shown in Figures 1a-1f.

It was also observed in Table 2 that, Attiéké samples collected from Adjame Bromokoute 1 and Adjame Bromokoute 2 had the highest number of fungi occurrence followed by Iwo Odoori, Iwo BHS, Ejigbo Ore meji collection with Ejigbo Beulah having the least growth. However studying these samples, the rate of occurrence of aflatoxigenic

Samples	Crude Protein (%)	Crude Fibre (%)	Crude Fat (%)	Ash (%)	Moisture Content (%)
A18	0.48f	1.10ab	0.18a	0.46bc	45.89e
B18	0.56e	1.12a	0.17ab	0.48ab	49.90b
A19	0.61d	1.08b	0.15bc	0.45c	43.83f
A20	0.69c	1.10ab	0.14c	0.47abc	46.49d
B19	0.71b	1.11a	0.15bc	0.49a	49.32c
B20	0.73a	1.08b	0.14c	0.49a	49.96a

Mean with the same letter in a column are not significantly different from each other at p>0.05

Table 1: Nutritional composition of wet attiéké sample.

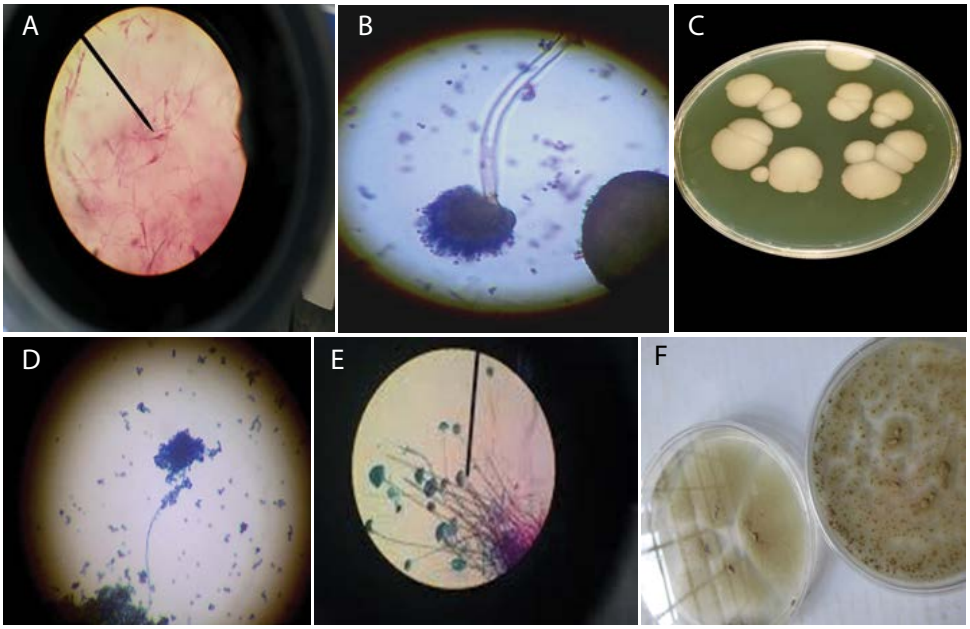

Figure 1: Bio-deteriorating fungal contents in 'Attieke' samples. **a:** Photomicrograph of *Penicillium chrysogenum.* **b:** Photomicrograph of *Aspergillus niger.* **c:** Pure culture of *Candida albicans* in PDA media plate. **d:** Photomicrograph of *Aspergillus flavus.* **e:** Photomicrograph of *Mucor hiemalis.* **f:** Pure culture of *Mucor hiemalis* in PDA media plate.

Fungal isolates	Ejigbo Ore meji	Adjame Bromokote 1	Iwo Odoori	Adjame Bromokote 2	Iwo BHS	Ejigbo Beulah
Penicillium chrysogenum	-	-	-	+	-	-
Aspergillus niger	+	+	+	+	+	+
Candida albicans	+	+	+	+	+	-
Aspergillus flavus	+	+	+	+	+	-
Mucor hiemalis	-	+	-	-	-	+

+ Presence of fungi; **-** Absence of fungi

Table 2: Occurrences of 'attieke' biodeteriorating fungi in different locations.

Sample	AFB_1 (µg/kg)	AFB_2 (µg/kg)	AFG_1 (µg/kg)
Adjame Bromokoute 1	1.64c	2.53b	1.51c
Ejigbo Ore meji	5.03b	2.46d	2.88a
Ejigbo Beulah	6.08a	2.48c	9.57f
Iwo BHS	6.12a	2.56a	1.44d
Adjame Bromokoute 2	6.47a	2.48c	1.43e
Iwo Odoori	6.72a	2.54b	1.95b

Mean with the same letter in a column are not significantly different from each other at P>0.05

Table 3: Aflatoxin concentrations in different 'Attiéké' samples.

Proximate Analysis	Crude Fibre	rude Fat	Ash	Moisture Content	Replicate	Sample
Crude Protein	-0.20	-0.80**	0.51*	0.38	0.01	0.95**
Crude Fibre		0.40	0.36	0.45	0.20	-0.32
Crude Fat			-0.22	-0.08	0.12	-0.80**
Ash				0.84**	0.08	0.46
Moisture Content					0.00	0.33
Replicate						0.00

*P<0.05=significant, **p<0.01=highly significant

Table 4: Correlation matrix of the nutritional composition of wet attiéké sample.

fungi (*Aspergillus* sp.) was higher in the order; Iwo Odoori>Iwo BHS>Adjame Bromokoute 2>Adjame Bromokoute 1>Ejigbo Ore meji>Ejigbo Beulah. In a contrasting situation, the occurrence of non-aflatoxic fungus was observed in the samples (Table 2).

The result shown in Table 3 shows the mean Aflatoxin concentrations of AFB_1, AFB_2 and AFG_1 in the studied Attiéké samples which are significantly different across each location (P<0.01), but AFB_1 found in samples from Ejigbo Beulah, Iwo BHS, Adjame Bromokoute 1 and Iwo Odo ori were not significantly different from one another, while the least AFB_1 was observed in sample from Adjame Bromokoute 2 (Table 3).

There were also no significant differences between the AFB_2 found in sample from Adjame Bromokoute 1 and Iwo Odoori but significantly different from sample from Ejigbo Beulah and Adjame Bromokoute 2 which are not significantly different from each other. The least AFB_2 concentration of Attiéké was obtained from sample collected from Adjame Bromokoute2. However, there were differences in all the AFG_1 concentration in the sample, the highest AFG_1 was obtained from sample Ejigbo Beulah, while the least AFG_1 was found in sample Adjame Bromokoute 2.

Results (Table 4) also show the correlation between all the proximate compositions in wet Attiéké. The crude protein is negative and strongly correlated with the crude fat (r=-0.80) and positively associated (r=0.50) with the ash content. There was negative and some correlation between the sample and Crude Fat (r=-0.80) while the sample is positive and strongly correlated with Crude Protein. Crude Fat shows a negative correlation and highly correlated with the sample while the ash content is positive and strongly correlated (r=0.84) with the moisture content of the sample.

Discussion

Results obtained on proximate compositions of food correlates with previous observations on 'Attiéké' [24]. A slight progression in moisture was recorded as the storage period increases as similarly reported, they opined that cassava dough and yam chips absorbed moisture during storage, and enhances the growth of fungi and other spoilage organisms [25,26,27]. The effect of moisture absorbed increases the water activity as a result of degrading activity of these fungi.

Furthermore, percentage crude fat, crude fibre observed decreased with storage period. This might be as a result of microbial degradation of nutritive substances in this food by certain fungi that coursed for their growth and development.

Lack of growth not until the third day could be the reductive effect of steam (heat) on the vegetative microorganisms as similarly reported, and not until then that emergence of spores that growth was recorded [28].

The variation in occurrence of fungi biomass across the samples could be as a result of different methods of handling which include processing and storage. *Penicillium chrysogenum* had been reported to be associated with damp or wet material which could be an indication of unhygienic water source [29]. *Mucor hiemalis* and *Candida albicans* observed in this study were also reported to be found from Cassava products such as; Attiéké, Fufu and Lafun. *A. flavus* and *A. niger* also characterized in this study were reported to be aflatoxigenic fungi found in Garri Aadun and in suya spices [30-34].

The aflatoxin concentrations across different locations were so high, though some authors reported a much higher level in dried cassava chips [35]. This could be due to deteriorated tubers predisposed to aflatoxin producing fungi which was similarly confirmed [36]. Also, the storage method of Cassava dough through refrigeration, left for days before final steaming (though the flavour, taste and color used to adjudge its quality were still pristine) also makes the food susceptible to fungal contamination and aflatoxin production. Studies in these locations further revealed that most of the production process involved in the making of Attiéké follows the usual traditional technologies. Sack container used in packing and pressing could be a reservoir of different fungal growth. The drying of Cassava dough on ground surfaces predisposes these to contamination from dust, fungi and other lamentable materials. Some reported that such practice enhanced association between the products and the soil which is the primary habitat of fungi [37]. Maize cobs were reported to have a much higher aflatoxin level when dried on ground though, this is worse in peeled cassava because the inherent protection in tuber will have been removed by peeling. Inherent protection in grain husks had been reported to safeguard rice and maize from aflatoxin contamination, fungi and weevils infestation [38-41].

Conclusion

The results of this study showed that major spoilage (biodeteriorating) fungi of Attiéké from Ejigbo, Iwo and Adjame in West Africa were mostly molds with *Aspergillus niger* and *A. flavus* having highest occurrence and *Candida albicans* and their percentage occurrence has direct effect on its food values. Obviously this food is distributed to other villages, towns, cities and countries notably Ife-Odan, Osogbo, Sekondi-Takoradi, this is an indication that if not properly managed consumers of this delicacy will be at risk of aflatoxicosis. Thus, modern technologies for hygienic storage mechanisms and proper sanitary measures are needed to be put in place. Adequate information concerning the level of fungi and aflatoxin contamination of this food and how to reduce the risks of exposure to aflatoxin during its processing and storage are needed to be conveyed to all level of society.

References

1. Terry ER, Doku EU, Arene OB, Mahungu NM (1983) Plantes racines tropicales: Culture et emplois en afrique.
2. Kohou F, Michel P (1988) Céréales en regions chaudes: Conservation et transformation. John libbey eurotext paris.
3. Kouadio NA, Angbo SF, Mosso K (1991) Etude Comparative, Méthodes de Préparations Traditionnelles de l'attiéké dans le sud de la Côte d'Ivoire. Industries Alimentaires et Agricoles 108: 703-706.
4. Kouassi B, Traoré A, Sirpé G (2008) Transformation et consommation des denrées alimentaires en Afrique de l'Ouest Centrale. Edition Katharina ISBN: 97828458697: 141-145.
5. Bendech A, Chauliac M, Rerolle GP, Kante N, Malvy DJ (2000) Les Enjeux du consommateur en milieu urbain a Bamako. Journal de Sante Publique 12: 45-63.
6. Giraud E, Brauman A, Kelele S, Gosselin L, Raimbault M (1995) Contrôle de la fermentation du manioc pour un meilleur gari: Utilisation d'un ferment de *Lactobacillus plantarum* à activité linamarase et amylase in Transformation alimentaire du manioc. Edition Orstorm Paris ISBN 2-7099-1279-1: 224-233.
7. Trèche S (1995) Importance du manioc en alimentation humaine dans différentes régions du monde. In: Transformation alimentaire du manioc. Edition Orstorm Paris 234-243.
8. FAO (2001) Production et exportation annuelles de manioc: Marche tropicaux. Food and Agricultural Oganisation Rome Italy 55: 170.
9. Essers S, Bosveld M, Van Der Grift RM (1993) Studies on the quantification of specific cyanogens in cassava products and introduction of a new chromogen. J Science Food Agri 63: 287-296.
10. Fortin J, Desmarais G, Assovie O, Diallo M (1998) L'Attiéké couscous de la Côte d'Ivoire. Le monde Alimentaire, pp: 2-6.
11. Mosso K, Aboua F, Gnakri D (2000) Détoxication en cyanure des aliments dérivés du manioc. Industrie Alimentaire et Agricole 117: 41-42.
12. Sahore DA, Nemlin GJ (2012) Changes in biochemical properties of fresh attiéké during its storage. Food Public Health 2: 99-103.
13. Djeni NT, N'Guessan KT, Dadier AT, Djè KM (2008) Impact of different levels of a traditional starter on the fermentation of cassava. Food Global Science Books 2: 10-13.
14. Aboua F (1998) Optimum conditions for cooking attiéké centre ivoirien de recherche technologique. J Trop Sci 38: 220-223.
15. Sotomey M, Ategbo EAD, Mitchipkpe EC, Gutierrez ML, Nago MC (2001) Innovation et diffusion des produits alimentaires en Afrique: l'attiéké au Bénin Savoir-faire et innovation en agroalimentaire en Afrique de l'Ouest. CIRAD 1-97.
16. Sargeant K, O'Kelly J, Carnaghan RBA, Allcroft R (1991) The assay of a toxic principle in certain groundnut meals. Vet Rec 73: 1219-1222.
17. Hamblin AM (2000) A focus on aflatoxin Smoked dried fishes samples stored for sale in contamination complication. Publication papers, p: 4.
18. Jimoh KO, Kolapo AL (2008) Mycoflora and aflatoxin production in market samples of some selected Nigerian food stuffs. Res J Microbiol 3: 169-174.
19. Malgorzata R, Loannou M (2012) Teaching Resources From Microbiology Laboratory. Hals Oer Project De Montfort University.
20. Huang B, Ham Z, Wub Y, Ren Y (2010) Simultaneous determination of aflatoxins B_1, B_2, G_1, M_1 and M_2 in peanut and their derivate product by ultra-high-performance liquid chromatography-tandem mass spectrometry. Anal Chim Acta 662: 62-68.
21. Cavaliere C, Foglia P, Guarino C, Nazzari M, Samperi R, et al. (2007) Determination of aflatoxins in olive oil by liquid chromatography-tandem mass spectrometry. Anal Chim Acta 596: 141-148.
22. Beltran E, Ibanez M, Sancho J, Cortes M, Yusa V, Hernandez F (2011) UHPLCMMS/MS highly sensitive determination of aflatoxion metabolite M1 and ochratoxion in a baby food and milk. Food Chem 126: 737-744.
23. AOAC (2005) Official methods of analysis of AOAC International 18th Eds association of official analytical chemists. Arlington Virginia USA 65: 1199-1221.
24. Aboua F (1989) Simple methods for evaluating attiéké spoilage in food chemistry. Trop Sci CIRT Abidjan 31: 313-317.
25. Jonathan SG, Ajayi L, Omitade Y (2011) Nutritional compositions, fungi and aflatoxins detection in stored 'gbodo' fermented (*Dioscorea rotundata*) and 'elubo ogede' fermented (*Musa paradisiaca*) from south western Nigeria. Afr J Food Sci 5: 105-110.
26. Gbolagade JS (2005) Bacteria associated with cultures of *Psathyrella atrombonata* (pegler) and *Schizophyllum commune* (fr.ex.fr) Nigerian edible mushrooms. Acta Phytopathologica Et Entomologica Hungarica 40: 333-340.
27. Jonathan SG, Adeniyi MA, Asemoloye MD (2016a) Nutrient value, fungal biodeterioration and aflatoxin contamination of suya spices a novel Nigerian indigenous snack. Hindawi - Scientifica 2016: ID 4602036.
28. Coulin P, Farah Z, Assanvo JB, Spillmann J, Puhan Z (2006) Characterization of the microflora of the fermented cassava product attiéké during traditional small scale preparation. Int J Food Microbiol 2: 131-136.
29. Samson RA, Honbraken J, Thrane U, Frisvad JC, Anderson B (2010) Food and indoor Fungi. CBS Laboratory Manual Series utrecht Netherlands, p: 390.
30. Kouamé AK, Djeni TN, N'Guessan FK, Dje MK (2012) Post-processing microflora of commercial Attiéké produced in the south of Côte d'Ivoire. Society for Appl Microb 56: 44-50.
31. Omafuvbe BO, Adigun AR, Ogunsuyi JL, Asunmo AM (2007) Microbial diversity in ready-to-eat fufu and lafun-fermented cassava products sold in Ile-Ife Nigeria. Res J Microb 2: 831.
32. Jonathan SG, Abdul-Lateef MB, Ayansina ADV (2013) Fungal and aflatoxin detection in fresh and stored 'garri ijebu' (locally processed food) *Manihot esculenta*. Report and Opinion 5: 13-19.
33. Jonathan SG, Adeniyi MA, Asemoloye MD (2015) Fungal biodeterioration, aflatoxin contamination and nutrient value of 'aadun'. Researcher 7: 26-32.
34. Jonathan SG, Asemoloye MD, Abe A, Olawuyi OJ, Aina D (2016b) Food value, fungi and aflatoxin detection in stored 'orunla' *Abelmoschus esculentus* L. (Moench) from Ibadan. Nigeria. Researcher 8: 7-18.
35. Kaaya NA, Warren HL (2005) A review of past and present research on aflatoxin in Uganda. Afr J Food Agric Nutr Dev 5:1.
36. Mestres C, Bassa S, Fagbohoun E, Nago M, Hell K (2004) Yam chip food sub-sector: Harzadous practices and presence of aflatoxins in Benin. J Stored Prod Res 40: 575-585.
37. Diener UL, Cole RJ, Sanders TH, Payne AG, Lee LS, et al. (1987) Epidemiology of aflatoxin foundation by *Aspergillus flavus*. Ann Rev Phytopathol 5: 249-270.
38. Hell K, Cardwell KF, Poehling HM (2003) Relationship between management practices, fungal infection and aflatoxin for stored maize in Benin. J Phytopathol 151: 690-698.
39. Kaaya AN, Kyamuhangire W, Kyamanywa S (2006) Factors affecting aflatoxin contamination of harvested maize in the three agro-ecological zones in Uganda. J Applied Sci 6: 2401-2407.
40. Udoh JM, Cardwell KF, Ikotun T (2000) Storage structures and aflatoxins content of maize in five agro-ecological zones of Nigeria. J Stored Prod Res 36: 187-201.
41. Liu Z, Gao J, Yu J (2006) Aflatoxins in stored maize and rice grains in Liaoning province China. J Stored Prod Res 42: 468-479.

This article was originally published in a special issue, **Advances in Microbiology and Biotechnology** handled by Editor(s). Dr. Gamil Sayed Gamil Zeedan, , National Research Center, Egypt