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

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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 AFB1 (1.03-6.72 µg kg⁻¹) and AFG1 (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 Ivoirian particularly the coastal population of the country [3]. Its appreciation is going beyond boundaries as a staple food due to black demand of consumers as shown below:

1. a) Ejigbo, Beulah Church (6°33'8''N, 3°18'26''E)
   b) Ejigbo, Oreo meji (6°30'8''N, 3°18'20''E)
2. a) Iwo, BHS (7°47'00''N, 4°12'00''E)
   b) Iwo, Odo, ori market (7°46'00''N, 4°12'00''E)
3. a) Adjame Bromokoute 1 (5.36°N, 4.02°W)
   b) Adjame Bromokoute 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

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:

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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 g.L\(^{-1}\)). 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 (AFB1), Aflatoxin B2 (AFB2) and Aflatoxin G1 (AFG1) [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 Rhome). This method is used after an extraction with acetonitrile and water, reaching limits of qualification between 0.012 and 0.073 µg kg\(^{-1}\).

**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 was weighed into a previously weighed crucible (W\(_{0}\)). The crucible plus sample (W\(_{1}\)) was 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 desicators, cooled for ten minutes and weighed (W\(_{2}\)) [23].

\[
\%\text{ Moisture} = \frac{W_1 - W_2}{W_0} \times 100
\]

**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 desicator and weighed [23].

\[
\%\text{ Ash} = \frac{\text{weight of ash}}{\text{Original weight of sample}} \times 100
\]

**Fibre determination**

2 g of the sample was weighed accurately into the fibre flask and 100 ml of 0.255 N H\(_2\)SO\(_4\) 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 desicator 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 desicator 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) and all analysis was computed 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 Attiévé 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.
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 mejie collection with Ejigbo Beulah having the least growth. However studying these samples, the rate of occurrence of aflatoxigenic

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

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.

<table>
<thead>
<tr>
<th>Fungal isolates</th>
<th>Ejigbo Ore mejie</th>
<th>Adjame Bromokote 1</th>
<th>Iwo Odoori</th>
<th>Adjame Bromokote 2</th>
<th>Iwo BHS</th>
<th>Ejigbo Beulah</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillium chrysogenum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mucor hiemalis</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

* Presence of fungi; - Absence of fungi

Table 2: Occurrences of ‘attiéké’ biodeteriorating fungi in different locations.
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 the making of Attiéké follows the usual traditional technologies. Sack container used in packing and pressing could be a reservoir of 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 Ijigbo, 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 Ile-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.
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