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

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Abstract

Derived foods from root and tuber crops, Attieké for example, are often consumed by African populace. Attieké 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 Attieké 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: Attieké; 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 Attieké. Attieké 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 appreciable is going beyond boundaries as a staple food due to black African-diaspora immigration [4]. In Cote d'Ivoire, Attieké plays a large part in household sustenance with regards to its combat against hunger and also its nutritional supplements [5]. Attieké is acknowledged to be representing 5% food expenditure and 20.5% Calories diet daily intake by Ivorian particularly the coastal population of the country [6]. Records shows that they were all steamed at the same time. A total of six (6) samples were collected from three (3) locations where ‘Attieké’ 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 mejii), (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 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.

The preparation of attieké 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 “F” 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 Attieké from Nigeria and Ivory Coast.

Materials and Methods

Collection of samples

Samples of prepared ready for cooking ‘Attieké’ (i.e., processed cassava dough) were randomly selected from two major table top food sellers in three (3) locations where ‘Attieké’ 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 mejii), (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 Bromokoute 2 (5.36°N, 4.02°W)

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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 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 (AFB₁), Aflatoxin B2 (AFB₂), Aflatoxin G1 (AFG₁) and Aflatoxin G2 (AFG₂) [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 emission wavelength was 425 nm. The ionization sources employed for the detection technique at the excitation wavelength of 362 nm and 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.H₂SO₄ 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 (Wₒ) 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 (W₁) [23].

\[
\text{% Dry Matter (DM)} = \frac{W_1 - W_0}{W_0} \times 100
\]

% 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 H₂SO₄ 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 W₁. The crucible with weight W₁ 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 weighed to obtain W₂. The difference W₁ – W₂ 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 Attiééké 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 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

### Table 1: Nutritional composition of wet attiéké sample.

<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 2: Occurrences of ‘attiéke’ biodeteriorating fungi in different locations.

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

* Presence of fungi; - Absence of fungi

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**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.
Aspergillus and Ash were recorded as the storage period increases as similarly reported, they are not significantly different from each other at P>0.05. The result shown in Table 3 shows the mean Aflatoxin concentrations of AFB<sub>1</sub>, AFB<sub>2</sub>, and AFG in the studied Attiéké samples. There were also no significant differences between the AFB concentrations in the sample, the highest AFG concentration was found in sample from Ejigbo Beulah, Iwo BHS, Adjame Bromokoute 1 and Iwo Odoori were not significantly different from one another, while the least AFB<sub>1</sub> was observed in sample from Adjame Bromokoute 2 (Table 3).

Table 3: Aflatoxin concentrations in different ‘Attiéké’ samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>AFB&lt;sub&gt;1&lt;/sub&gt; (µg/kg)</th>
<th>AFB&lt;sub&gt;2&lt;/sub&gt; (µg/kg)</th>
<th>AFG&lt;sub&gt;1&lt;/sub&gt; (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjame Bromokoute 1</td>
<td>1.64c</td>
<td>2.53b</td>
<td>1.51c</td>
</tr>
<tr>
<td>Ejigbo Ore mej</td>
<td>5.03b</td>
<td>2.48d</td>
<td>2.88a</td>
</tr>
<tr>
<td>Ejigbe Beulah</td>
<td>6.08a</td>
<td>2.48c</td>
<td>9.57f</td>
</tr>
<tr>
<td>Iwo BHS</td>
<td>6.12a</td>
<td>2.56a</td>
<td>1.44d</td>
</tr>
<tr>
<td>Adjame Bromokoute 2</td>
<td>6.47a</td>
<td>2.48c</td>
<td>1.43e</td>
</tr>
<tr>
<td>Iwo Odoori</td>
<td>6.72a</td>
<td>2.54b</td>
<td>1.95b</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Proximate Analysis</th>
<th>Crude Protein</th>
<th>Crude Fibre</th>
<th>crude Fat</th>
<th>Ash</th>
<th>Moisture Content</th>
<th>Replicate</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjame Bromokoute 1</td>
<td>-0.20</td>
<td>0.51</td>
<td>0.38</td>
<td>0.01</td>
<td>0.95*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ejigbo Ore mej</td>
<td>-0.80*</td>
<td>0.36</td>
<td>0.45</td>
<td>0.20</td>
<td>-0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjame Bromokoute 2</td>
<td>-0.22</td>
<td>-0.08</td>
<td>0.12</td>
<td>0.84</td>
<td>0.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iwo BHS</td>
<td>0.00</td>
<td>0.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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