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 AFB1 (1.03-6.72 µg kg\(^{-1}\)), AFB2 (2.46-2.56 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 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 "Ba" 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 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 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...
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) 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 350B1 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. The coupling of HPLC to mass spectrometry was also used for the detection technique at the excitation wavelength of 362 nm and was used. The coupling of HPLC to mass spectrometry was also used for the extraction technique at the excitation wavelength of 362 nm and was also used.

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 W₀. The crucible with weight Wₐ 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 W₂ - Wₐ gives the weight of fibre [23].

% Fibre = \( \frac{W₂ - Wₐ}{\text{Weight of sample}} \times 100 \)

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 Attié ké 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...
The 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 processing and storage.

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

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>AFB, (µg/kg)</th>
<th>AFB, (µg/kg)</th>
<th>AFG, (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>Ejigbo 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 3:** Aflatoxin concentrations in different ‘Attiéké’ samples.

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

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<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></td>
<td>-0.20</td>
<td>-0.80°</td>
<td>0.51°</td>
<td>0.38</td>
<td>0.01</td>
<td>0.95°</td>
<td></td>
</tr>
<tr>
<td>Crude Fibre</td>
<td>0.40</td>
<td>0.36</td>
<td>0.45</td>
<td>0.20</td>
<td>-0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude Fat</td>
<td>-0.22</td>
<td>-0.08</td>
<td>0.12</td>
<td>0.84°</td>
<td>-0.80°</td>
<td></td>
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<tr>
<td>Ash</td>
<td>0.00</td>
<td>0.33</td>
<td>0.46</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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


