



Research Article

Assessment of Aflatoxin Levels in Akara Processed from Bambara Groundnut and Cowpea Seeds

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Abstract

Aflatoxins (AFs) are toxic metabolites produced by different species of toxigenic fungi called mycotoxins, they are natural contaminants in raw foods and feeds, especially legumes and cereals. Aflatoxin contamination of food is a major health risk due to its carcinogenic and hepatotoxic properties. The objective of this study was to determine and compare aflatoxin concentrations, nutritional and sensory evaluation of akara samples made from 100% cowpea paste (A1), 100% bambara groundnut (BGN) paste (A5), and from bambara groundnut-cowpea composite paste mixtures (A2, A3, A4); to assess the products' safety for consumption. Macro and micro mineral concentrations, sensory evaluation of the samples were also assessed. Five akara samples A1, A2, A3, A4, A5 were made, The highest AFs concentration (0.070 µg/Kg) was found in A5 (Akara from BGN paste only) while the least concentration (0.027 µg/Kg) was found in A1 (Akara from cowpea paste only). A5 had highest micro and macro mineral nutrients while A1 had the least. All akara samples were well accepted by consumers. Aflatoxin concentrations in akara samples (A1-A5) were much lower than the standard safe level of aflatoxin in food. Hence, consumption of akara made from bambara groundnut paste (A5), cowpea paste (A1) and composite mixture of both (A2, A3, A4) is safe as it relates to aflatoxin B1.

Keywords

Aflatoxin, Bambara Groundnut, Cowpea, Akara, Mineral Nutrients, Sensory Evaluation

1. Introduction

Bambara groundnut is one of Africa's most important legumes. It is a sustainable, and low-cost source of complex carbohydrates, plant-based protein, unsaturated fatty acids, and essential minerals like Magnesium, Iron, Zinc, and Potassium. As a legume, it also helps to improve soil fertility by fixing atmospheric nitrogen into the soil. There is a growing trend about increasing the consumption and in-take of plant-based

diets, due to their importance in human nutrition and health; thereby increasing the need for the production of more plant-based protein foods. One of the crops that fit perfectly for this demand is bambara groundnut. This nut serves as a very important source of the essential nutrients like protein, especially in areas that animal sources of protein are lacking. Bambara groundnut seed is used to make nutritious food because the

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proximate analyses showed that it gives a near perfect balanced diet even when taken alone [1]. On the average, proximate analysis revealed that bambara groundnut seeds have 65.0% carbohydrate, 25.0% protein, 7.5% fat, and 6.0% fiber; therefore, this nutrient-dense legume can be termed a “complete food” [2]. It was also found to be rich in vitamins like Vitamin A, Vitamin B2, Vitamin B3, and carotene [3]. The seeds of bambara groundnut is rich in essential amino acids which include methionine, leucine, isoleucine, lysine, phenylalanine, threonine, valine and tryptophan [4]. Apart from its numerous nutritional qualities, Bambara groundnut also serves medicinal purpose. It is used in the treatment of diarrhoea, anaemia, abscesses, internal injuries, ulcers, infected wounds, epilepsy, cataracts, and menorrhagia during pregnancy and nausea in pregnant women [5]. However, bambara groundnut seeds have also been documented to be susceptible to contamination by aflatoxins [6]. Hence there is need to evaluate the safety of bambara groundnut based food products for human consumption. This work aims at determining and comparing aflatoxin concentrations in akara made from bambara groundnut (BGN) paste only, bambara groundnut-cowpea composite paste mixtures, in comparison to akara made from cowpea paste only in order to assess the products’ safety for consumption.

2. Materials and Methods

2.1. Cowpea Paste Preparation

Seeds were soaked in water in a ratio of 1:4 (seed: water). After soaking for 10 minutes, cowpea seeds were manually dehulled by rubbing the seeds between the palms until complete removal of the seed coat was achieved and the creamy colour of the cotyledon was seen. More water was poured into the beans for easy floating and decanting of the seed coats, this process was repeated until the bean was clean and no seed coat remained in the water. The de-hulled cowpea seeds were allowed to get soaked in the water for another 10 minutes, to allow it soften. The beans were drained and transferred into an electric blender (vitamix 1002), water was added to give moisture of about 40% (wet basis), and blender was set on high speed. Cowpea seeds were blended for 5 minutes.

2.2. Bambara Groundnut Paste Preparation

Seeds of bambara groundnut were soaked in water in a ratio of 1:8 (w/v) for seed:water, and left overnight. Bambara groundnut seeds were dehulled on the kitchen sink railings, using a flat wooden spatula. The dehulled seeds were then poured into a bowl and water was poured over for easy floating and decanting of the seed coats, this process was repeated until we had clean seeds with no seed coat. The de-hulled bambara groundnut seeds were drained and transferred into an electric blender (vitamix 1002), water was added to give moisture of about 60% (wet basis), and blender set on high speed

was allowed to blend the beans for about 5 minutes.

2.3. Sample Preparation

Bambara groundnut paste (whole), cowpea paste (whole), and composite mixtures of cowpea/bambara groundnut pastes were made as in Table 1 below.

Table 1. Composition of pastes used for akara.

Sample/code	Composite ratio (cowpea: bambara)
A1	1:0
A2	3:1
A3	2:1
A4	1:1
A5	0:1

2.4. Akara Preparation

Akara was prepared by the method of [7] with slight modifications. 400g of paste (Table 1 above) was used for each sample. Each batch of paste was whipped manually for 3 minutes using a wooden ladle, this helped to incorporate air into the paste, 1g of diced scotch bonnet pepper (ata rodo), 1g of diced onions and a pinch of salt was added to the paste and the mixture was whipped for another 2 minutes. Paste portions were scooped into a spoon and dropped into a pot of hot oil ($180 \pm 2^\circ\text{C}$). Frying continued until golden brown colour was attained. Total frying time per batch was 5-6 minutes. Akara balls were removed from the oil and put inside a colander lined with parchment paper, to drain excess oil. Some akara was kept warm for sensory evaluation, while others were allowed to cool and put in a labeled plastic container for laboratory analyses.

2.5. Sensory Evaluation of Akara Samples

Akara samples were coded and served to a 20-man trained panelist, familiar with akara. Evaluation was done using 9-point hedonic scale where 9 was maximum (like extremely) and 1 was minimum (dislike extremely). Samples were scored for colour, fluffiness, taste, aroma and overall acceptability. The panelists were instructed to rinse their mouth before testing the next sample, to prevent taste interference.

2.6. Mineral Analyses of Akara Samples

2.6.1. Determination of Calcium, Potassium and Sodium Concentrations in Akara Samples

Determination of calcium, potassium and sodium concentration was done by the method of [8]. 2 M HCl (5 ml) was

added to the ash of the sample for digestion. This was followed by heating to dryness on a heating mantle. Another 5 ml of 2 M HCl was added to the dried digested sample; this was brought to boil and then filtered into a 100 ml volumetric flask, using Whatman No 1 filter paper. The filtrate was made to mark with distilled water, stoppered and made ready for reading of concentration on the Jenway Digital Flame Photometer (PFP7 model) using the standard corresponding to each mineral element. Different concentrations of calcium chloride (CaCl_2), potassium chloride (KCl_2) and sodium chloride (NaCl) were used as standards for the mineral elements calcium (Ca), potassium (K) and sodium (Na) determined respectively. The concentration of each of the mineral element was calculated using the formula:

$$\text{Mineral element (mg/kg)} = \frac{\text{MR} \times \text{slope} \times \text{dil.Factor}}{100} \quad (1)$$

where MR = Meter reading

2.6.2. Determination of Phosphorus Concentration in Akara Samples

Phosphorus was determined by the vanado-molybdate spectrophotometric method as described by [8]. Ash was treated with 2 M HCl solution as described for calcium, potassium and sodium concentration above. 10 ml of the filtrate was pipetted into 50 ml standard flask and 10 ml of vanado-molybdate solution was added, the flask was made up to mark with distilled water, stoppered and left for 10 minutes for full yellow development. The concentration of phosphorus was obtained by taking the optical density (OD) on a Metrohm spectronic 21D spectrophotometer at a wavelength of 470nm.

The concentration of phosphorus was calculated as:

$$\text{Phosphorus (mg/kg)} = \frac{\text{Absorbance} \times \text{slope} \times \text{dil.Factor}}{100} \quad (2)$$

2.6.3. Determination of Micro-minerals-iron, Zinc, Copper, Selenium, Manganese Concentration in Akara Samples

The micro mineral elements (iron, zinc, copper, selenium, manganese) were analyzed as described by [9]. Different concentrations of the chloride salts of each mineral element were prepared. Optical density of each standard solution was measured at 540 nm. Graph of absorbance against concentration was obtained for the salt of each micro-mineral, the slope was found for each graph. The diluent of digested ash sample of BGNC obtained above was aspirated into the Buck 200 atomic absorption spectrophotometer through the suction tube provided, and optical density of the sample was also read at 540nm. The corresponding concentration of each micro mineral element in the sample was taken as follows:

$$\text{Mineral element (mg/kg)} = \frac{\text{Absorbance} \times \text{slope} \times \text{dilution factor}}{100} \quad (3)$$

2.7. Extraction, Detection and Quantification of Aflatoxin B1 Using Thin Layer Chromatographic (TLC) Techniques

This was done by the method of [10].

2.7.1. Extraction

1 g of sample was weighed into a 100ml conical flask, 2.5 ml of distilled water and 25 ml of chloroform was added. The flask was stoppered and shaken on a shaker for 30 minutes after which the solution obtained was filtered using a Whatman No. 1 filter paper. 10ml of each extract or filtrate was collected and evaporated to dryness to a volume of 5ml on a hot water bath.

2.7.2. Detection

Chloroform (1 ml) and 0.2 ml of the reconstituted extract was spotted on a precoated 20 x 20cm TLC plate along with aflatoxin standards of known concentration. The spotted TLC plate was developed in an equilibrated tank containing chloroform: acetone (9:1 v/v). The developed TLC plate was air-dried at ambient temperature ($28 \pm 2^\circ\text{C}$) and aflatoxins were detected under UV light at wavelength of 360nm. A colour change from blue to yellow upon exposure to aqueous sulphuric acid (50:50 v/v) confirms the presence of Aflatoxin B1.

2.7.3. Quantification

0.5 μm thick preparative TLC plates will be employed for the quantitation. 0.8ml of stored extract above after aflatoxin extraction will be applied to the plate as band rather than a spot to chromatograph the maximum amount of sample at the same time. The preparative TLC plates will be developed in an equilibrated tank as in aflatoxin extraction. When the solvent front rises to about $\frac{3}{4}$ of the total length of the plate, the plate will be taken out of the tank and examined under UV light. Once the area containing the toxin of interest is located after ultra-violet (UV) light examination, it will be scrapped off, eluted with chloroform and filtered using Whatman No. 1 filter paper. The extract will be evaporated to dryness over a hot water bath and reconstituted with 3ml Chloroform. The 3ml reconstituted solution and aflatoxin standard of 20 $\mu\text{g/ml}$ concentration will be used to read Absorbance or Optical Density on an ultraviolet Spectrophotometer (Cecil Instrument CE505) at a wavelength of 360nm.

Aflatoxin concentration in $\mu\text{g} / \text{kg}$ was calculated using the formula:

$$\text{Aflatoxin conc. } (\mu\text{g/kg}) = \frac{\text{Absorbance of sample} \times \text{conc. of standard} \times \text{dil. Factor}}{\text{Absorbance of standard}} \quad (4)$$

2.8. Statistical Analyses

Experiments were conducted in triplicates, except for the sensory evaluation which had 20 replicates. SPSS (version 20)

was used to analyse the data. Values were expressed in Means $\pm$ standard error of means (SEM). Mean values were assessed by one-way analysis of variance (ANOVA), means were separated by Duncan's multiple range test (DMRT). Threshold for significance was set at $p < 0.05$.

3. Results and Discussion

3.1. Aflatoxin B1 Concentrations in Akara Samples

Table 2. Aflatoxin B1 concentrations in akara samples.

SAMPLE CODES	CP: BG Ratio	AFLATOXIN B1 ($\mu\text{g/Kg}$)
A1	1:0	0.027 $\pm$ 0.001e
A2	3:1	0.037 $\pm$ 0.001d
A3	2:1	0.050 $\pm$ 0.001b
A4	1:1	0.043 $\pm$ 0.001c
A5	0:1	0.070 $\pm$ 0.001a

Values are means of three determinations $\pm$ standard error of means (S.E.M).

Means with different alphabets within the same column are significantly different at $P < 0.05$.

A1= Akara made from cowpea paste only (ratio 1:0, CP: BP)

A2= Akara made from composite mixture of cowpea paste and bambara groundnut paste (ratio 3:1, CP: BP)

A3= Akara made from composite mixture of cowpea paste and bambara groundnut paste (ratio 2:1, CP: BP)

A4= Akara made from composite mixture of cowpea paste and bambara groundnut paste (ratio 1:1, CP: BP)

A5= Akara made from bambara groundnut paste only (ratio 0:1 CP: BP)

Cowpea and bambara groundnut seeds are prone to aflatoxin contamination during storage and late harvest, especially when exposed to warmth and humidity. Table 2 shows the aflatoxin B1 concentrations in akara samples. The highest concentration (0.070 $\mu\text{g/Kg}$) was found in A5 while the least concentration (0.027 $\mu\text{g/Kg}$) was found in A1. This result suggests differences in seed susceptibility to aflatoxin attack. Bambara groundnut grows underground, with a thicker seed coat, while cowpeas grow above the ground and have a softer seed coat; thereby bambara groundnut seeds may have been more exposed to aflatoxins producing microbes like *Aspergillus flavus* and *Aspergillus parasiticus* during growth, drying and storage in comparison to cowpeas. The raw bambara groundnut seed flour used had been reported earlier to be 0.81 $\mu\text{g/kg}$ [11] representing a marked reduction relative to the 1000 $\mu\text{g/kg}$ previously reported by [12]. This indicated that

the bambara seeds used in this research were well dried and well stored. The concentration of aflatoxin in the akara sample from bambara groundnut seeds only was 0.07 $\mu\text{g/kg}$. It is noteworthy that processing the bambara into akara had a reducing effect on the aflatoxin level. Research has demonstrated that food processing techniques that uses high temperature (150°C- 200°C) reduces Aflatoxin B1 concentration by at least 40 percent [13, 14]. Studies by [15] stated specifically that frying helps in the reduction of aflatoxin B1 because aflatoxins are heat labile and undergo pyrolytic degradation at elevated temperatures in the presence of other food matrix components, resulting in decomposition to non-toxic metabolites. There was reduction in the level of aflatoxin in varied proportions from composites samples A2, A3, and A4; however, the reduction in aflatoxin concentration did not follow any specific pattern.

3.2. Macro and Micro Mineral Nutrients in Akara Samples

Table 3. Concentration of mineral nutrients (mg/100g) in akara samples.

SAM- PLES	Zn	Fe	Cu	Se	Mn	Ca	K	Na	P
A1	1.36 ±0.02e	1.28 ±0.02e	3.30 ±0.02e	0.002 ±0.00e	1.74 ±0.02d	7.97 ±0.03e	1.49 ±0.03e	8.14 ±0.02e	92.8 ±0.02e
A2	1.47 ±0.02d	1.41 ±0.02d	4.58 ±0.01d	0.03 ±0.00d	1.88 ±0.02c	8.23 ±0.03d	1.53 ±0.01d	8.38 ±0.01d	94.3 ±0.00d
A3	1.58 ±0.01b	1.58 ±0.01b	5.20 ±0.02c	0.05 ±0.00b	2.01 ±0.04b	8.74 ±0.02b	1.59 ±0.04b	8.54 ±0.01b	97.2 ±0.01b
A4	1.52 ±0.01c	1.47 ±0.01c	6.06 ±0.02b	0.04 ±0.00c	1.94 ±0.01c	8.42 ±0.02c	1.56 ±0.01c	8.43 ±0.01c	96.1 ±0.02c
A5	1.66 ±0.02a	1.71 ±0.02a	6.15 ±0.02a	0.06 ±0.00a	2.11 ±0.02a	8.93 ±0.04a	1.62 ±0.01a	8.61 ±0.00a	98.5 ±0.03a

Values are means of three determinations ± standard error of means (S.E.M).

Means with different alphabets within the same column are significantly different at $P < 0.05$.

A1= Akara made from cowpea paste only (ratio 1:0, CP: BP)

A2= Akara made from composite mixture of cowpea paste and bambara groundnut paste (ratio 3:1, CP: BP)

A3= Akara made from composite mixture of cowpea paste and bambara groundnut paste (ratio 2:1, CP: BP)

A4= Akara made from composite mixture of cowpea paste and bambara groundnut paste (ratio 1:1, CP: BP)

A5= Akara made from bambara groundnut paste only (ratio 0:1 CP: BP)

Akara sample from bambara paste only (A5) had the highest concentrations for all the mineral-nutrients determined, while akara sample from cowpea paste only (A1) had the least concentrations. Mineral-nutrients are needed in small quantities in the body, however, their impact on the body's health is critical, and deficiency in any of them can cause severe and even life-threatening conditions. One way to assess the rich micronutrients in bambara groundnut seeds is to make it into akara. Akara samples from composite pastes of bambara and cowpea (A2, A3, A4) contained more micronutrients than the one made from cowpea paste alone (A1). This result aligned with the report of [16] that mixed grain products are more nutrient-dense than single grain products. The observed higher mineral content in sample A3 compared to

sample A2 was contrary to the expected trend, based on the mineral composition of akara made from the individual legumes A1 (least mineral concentrations) and A5 (highest mineral concentrations). This suggests that mineral retention in composite legume products is not a function of dilution only, but may be influenced by other intrinsic factors during food processing. A plausible explanation is the synergistic effect of legumes. In this case, the ratio 2:1 composite mixture for akara (sample A3) may have provided the optimal CP: BP ratio, which improved extraction of minerals during laboratory analysis, while ratio 1:1 composite mixture for akara sample A4 may have passed the optimal stage and caused precipitation, which led to lower mineral concentration readings.

3.3. Sensory Properties of Akara Samples

Table 4. Sensory evaluation of akara samples.

SAMPLE	COLOUR	FLUFFINESS	AROMA	TASTE	OVERALL ACCEPTABILITY
A1	7.7 ±0.2a	7.4± 0.3b	7.9± 0.1a	7.4± 0.1ab	7.9± 0.1a
A2	7.3±0.1c	7.8±0.2a	7.7±0.29	7.2±0.1b	7.9±0.1a
A3	7.7±0.2a	7.7±0.1a	7.9±0.2a	7.6±0.2a	7.6±0.1b
A4	7.6±0.1b	7.9±0.1a	7.8±0.2a	7.7±0.3a	7.8±0.2a

SAMPLE	COLOUR	FLUFFINESS	AROMA	TASTE	OVERALL ACCEPTABILITY
A5	7.5±0.1b	7.6±0.1a	7.4±0.1b	7.8±0.1a	7.9±0.1a

Values are means of twenty determinations ± standard error of means (S.E.M).

Means with different alphabets within the same column are significantly different at $P < 0.05$.

A1 = Akara made from cowpea paste only (ratio 1:0, CP: BP)s

A2 = Akara made from composite mixture of cowpea paste and bambara groundnut paste (ratio 3:1, CP: BP)

A3 = Akara made from composite mixture of cowpea paste and bambara groundnut paste (ratio 2:1, CP: BP)

A4 = Akara made from composite mixture of cowpea paste and bambara groundnut paste (ratio 1:1, CP: BP)

A5 = Akara made from bambara groundnut paste only (ratio 0:1 CP: BP)

All akara samples were acceptable to the panel of evaluators. This result agrees with what was reported by [17] on the sensory evaluation of akara produced from bambara and cowpea flour blends. It is interesting to note that the score for overall acceptability of akara made from cowpea paste alone (control-A1) was the same with the score for the akara sample made from bambara groundnut paste alone. This indicates that bambara groundnut seeds will compete well with cowpea seeds in the production of akara; hence relieving the dependence on cowpea and improving the house hold utilization of bambara groundnut, an underutilised legume in Southwest, Nigeria.

4. Conclusions

The safety of bambara based akara for human consumption was validated in this study. Also, the possibility of making akara from bambara groundnut seeds paste and composite mixture of cowpea paste / bambara groundnut paste was established in this study, with the optimal ratio being ratio 2:1, CP: BP.

Abbreviations

BGN	Bambara Groundnut
BP	Bambara Paste
CP	Cowpea Paste
CP:BP	Cowpea Paste to Bambara Paste Ratio
AF	Aflatoxin
AFs	Aflatoxins
TLC	Thin Layer Chromatography
UV	Ultra-violet

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Author Contributions

Olanipekun Oyeyoyin Taiwo: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing

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Conflicts of Interest

The authors declare no conflicts of interest.

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