Fungal Biodeterioration, Aflatoxin Contamination, and Nutrient Value of “Suya Spices”

Segun Gbolagade Jonathan, Mary Adejoke Adeniyi, and Michael Dare Asemoloye

Food & Environmental Mycology/Biotechnology Unit, Department of Botany, University of Ibadan, Ibadan 200284, Oyo State, Nigeria

Correspondence should be addressed to Michael Dare Asemoloye; asemoloyemike@gmail.com

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This work aimed to analyze the nutrient values, examine the biodeteriorating fungi biota, and analyze the mycotoxin contents of “Suya spices.” Fungi with highest percentage occurrence on all the samples are Aspergillus niger, Aspergillus flavus, Aspergillus parasiticus, Aspergillus ochraceus, Fusarium sp., Rhizopus stolonifer, yeast, and Trichoderma koningii. Nutrient composition of the samples is significantly different statistically ($P < 0.05$) with high protein (9.53% to 13.17%), fiber (9.27 to 13.17%), carbohydrate (46.27% to 50.90%), and ash (8.47% to 9.70%) contents but low moisture (9.03% to 9.47%) and fat (9.77% to 13.53%) contents.

Aflatoxin analysis of the samples revealed that they all contain aflatoxin in varying amount but no detectible aflatoxin content in the control. 59.54% of the detected aflatoxin is aflatoxin B$_1$ with highest recorded in Agbowo, Mokola, and Sango samples (i.e., 28.03, 22.44, and 13.8 $\mu$g/kg, resp.). 4.78% of the aflatoxin is aflatoxin B$_2$ which is only found in Sango and Mokola samples (3.59 and 2.6 $\mu$g/kg, resp.). 32.76% of aflatoxin is aflatoxin G$_1$ with the highest found in Agbowo and Mokola samples (i.e., 18.63 and 10.41 $\mu$g/kg, resp.). 2.93% of the aflatoxin is aflatoxin G$_2$ which is only detected in Sango and Agbowo samples (i.e., 1.19 and 2.65 $\mu$g/kg, resp.).

1. Introduction

“Suya spices” are a Nigerian indigenous spice commonly used on roasted meat (barbecued meat) to give it a unique desired taste and it originated from the Hausa speaking people in the country. It is the special blend of peppers and spices that is used to make Nigerian Suya (Nigerian shish kebab, roasted skewered meat with a particularly African twist). The spice consists of ground pepper ($Capsicum$ sp.), $Xylopia$ aethiopica, $Piper$ guineense, and $Monodora$ myristica [1]. These spices help in adding aroma and flavor to the barbecued meat (Suya). Though Suya is prepared by the Hausas, its consumption transcends the borders of ethnicity, especially among the elites during relaxation period. Early studies showed that spices used in the preparation of Suya may contain high population of bacteria and fungi, which remain viable even at the time of marketing [2, 3], also according to [4]. If the spice is not hygienic or not properly kept, it may be contaminated by microorganism and may cause health hazard conditions like food poisoning. Large groups of microorganism have been isolated from some spices and some vended foods of which fungal groups are notable. Despite the fact that some spices and herbs have been documented to have antimicrobial activities, the quantity of added spices to food may not be enough to adequately inhibit microbial contaminations most especially fungi; even the antimicrobial activities of these spices may vary widely depending on the type of spices [5].

Microbial contamination has been reported to be the cause of food illnesses and spoilage [6–8] and many of these microbes are fungi. Fungi are ubiquitous or cosmopolitan (i.e., they can be present everywhere, in the air, water, and soil, and even on man and inside him) as explained by Jonathan et al. [8]. They are group of organisms known to be good “biodegraders” of waste, many of which have different characteristic mode of converting waste of living and dead tissues of various products such as plants products, agricultural products, wood and paper products, dead animal...
2. Materials and Methods

2.1. Sources of Materials for “Suya Spice” Production. Pepper (Capsicum sp.), Piper guineense, Maggi, and salt were purchased from Bodija market, Ibadan, Nigeria.

2.2. Collection of “Suya Spice” Samples. Five samples of “Suya spices” (500 g each) were purchased from different locations in Nigeria. These locations are known to be famous in Nigeria, well-populated, and active in marketing activities. These locations are Agbowo, Sabo, Mokola, Ojoo, and Sango. Also a laboratory sample was used as control for each of “Suya spices.”

2.3. Preparation of Laboratory Sample. Dry pepper (Capsicum sp.) and Piper guineense were ground to fine powder and were mixed with Maggi and salt.

2.4. Isolation of Fungi Biota. The samples were brought to the department and each sample was homogenized and 1 g was dissolved in 10 ml distilled water and then 1 ml of the mixture was added to 9 ml of sterilized distilled water followed with serial dilution at 10⁻¹ to 10⁻⁶, 1 ml of dilution 10⁻⁶ inoculated into the medium using direct inoculation method. The medium used for the isolation was potato dextrose agar (PDA) in plate and incubated at 30 ± 2°C for 5 to 7 days according to the procedure described by Jonathan and Olowolafe [21]. The cultures were examined under microscope for fruiting bodies and hyphae to determine the presence of fungi.

2.5. Characterization and Identification. Characterization and identification of isolated fungi were done based on the morphological characters and microscopic structures of the fungi [6, 7, 13]. The colonies of the organism were observed for peculiar characteristic colonial morphology and this was done using the following listed features:

(i) Colonial appearance.
(ii) Rate of growth followed at regular intervals.
(iii) Texture of colonies.
(iv) Colour of colonies.
(v) Reverse side or colour of underside.

Microscopic morphology and type of asexual spores produced were studied through use of photomicrograph and identified by reference to the compendium of soil fungi [22].

2.6. Proximate Analysis. Samples of Aadun were taken for proximate analysis and the determination of various parameters was carried out at KAPPA laboratories, Ibadan. The moisture, crude protein, crude fat, crude fiber, and total ash were determined using AOAC [23] methods while the carbohydrate was determined by difference.

2.7. Aflatoxin Analysis. The aflatoxin analysis was carried out in pathology laboratory of International Institute of Tropical
Agriculture (IITA), Ibadan, using the HPLC methods as described by Oluwafemi and Ibeh [24]. The HPLC is made up of LDC, with Milton Roy, Constametric 1 pump, and a Lichrosorb RP-18 column (Merck Hibar) with particle size of 5 μm, length of 125 mm, and inside diameter of 4 mm. The pump pressure is 60 MPa and the injector was of an automatic type (Rheotype Gilson Abimed Model 231). The detector had a fluorescence spectrophotometer (Shimadzu RF 535, gamma excitation 365 mm, and gamma emission 444 nm) and the flow rate was 1 mL per minute and the injection volume was 50 μL with the use of mobile phase containing water/acetonitrile (75:25) with flow rate 1.2 mL min⁻¹ for 20 min.

50 gram of each sample of “Suya spice” was defatted by extraction with N-hexene Soxhlet-type extractor and the defatted residue was extracted with ethyl acetate (three times, 50 mL/each). The extracts were combined, dried over anhydrous sodium sulphate, filtered and then concentrated under vacuum to near dryness, transferred into brown glass vial, and evaporated under nitrogen stream. For cleaning up the crude extracts, the crude extract was suspended in 1 mL chloroform and applied to 14 × 0.8 cm column containing 2.5 Kiesel gel 60 and 70/230 silica gel. The aflatoxin analysis was done using Lichrosorb RP-18 column. The quantitative determination of the aflatoxins was carried out compared with standard aflatoxin B₁ (Sigma).

3. Result and Discussion

The fungal isolates from samples of “Suya spices” are mostly of filamentous fungi most of which belong to the species Aspergillus. The fungi that appear mostly on the vended samples (i.e., are isolated from all the market samples) are Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Aspergillus parasiticus, Fusarium sp., Rhizopus stolonifer, yeast, and Trichoderma koningii (Figures 1(a)–1(l) with their
Table 1: Proximate analysis of “Suya spices.”

| Location | Moisture content (%) | Protein content (%) | Fat content (%) | Ash content (%) | Fiber content (%) | Carbohydrate content (%) |
|----------|----------------------|---------------------|----------------|----------------|-------------------|--------------------------|
| Control  | 9.10<sup>c</sup>     | 9.53<sup>c</sup>    | 11.17<sup>c</sup> | 8.77<sup>c</sup> | 13.17<sup>d</sup> | 48.30<sup>b</sup>       |
| Agbowo   | 9.73<sup>a</sup>     | 10.80<sup>e</sup>   | 12.47<sup>b</sup> | 9.70<sup>c</sup> | 9.77<sup>c</sup>   | 47.53<sup>c</sup>       |
| Sabo     | 9.03<sup>c</sup>     | 9.63<sup>e</sup>    | 13.53<sup>a</sup> | 9.79<sup>c</sup> | 9.27<sup>d</sup>   | 50.60<sup>a</sup>       |
| Mokola   | 9.43<sup>b</sup>     | 13.17<sup>a</sup>   | 10.53<sup>d</sup> | 9.37<sup>e</sup> | 11.30<sup>e</sup>  | 46.27<sup>d</sup>       |
| Ojoo     | 9.47<sup>b</sup>     | 12.33<sup>d</sup>   | 10.17<sup>c</sup> | 9.30<sup>e</sup> | 12.43<sup>e</sup>  | 46.30<sup>d</sup>       |
| Sango    | 9.17<sup>c</sup>     | 11.20<sup>f</sup>   | 9.77<sup>f</sup>  | 8.47<sup>d</sup> | 10.50<sup>d</sup>  | 50.90<sup>d</sup>       |

Each value is a mean of three replicates. Values in the same column with different letters as superscripts are significantly different by Duncan multiple range test ($P \leq 0.05$).

Table 2: Aflatoxin concentration of “Suya spices” samples collected from locations in Western Nigeria.

(a)

| Location | AFB1 (μg/kg) | AFB2 (μg/kg) | AFG1 (μg/kg) | AFG2 (μg/kg) | Total (μg/kg) |
|----------|--------------|--------------|--------------|--------------|---------------|
| Control  | 0            | 0            | 0            | 0            | 0             |
| Sango    | 13.8         | 3.59         | 8.66         | 0.34         | 27.24         |
| Agbowo   | 28.03        | 0            | 18.63        | 2.65         | 49.31         |
| Ojoo     | 3.85         | 0            | 0            | 0            | 3.85          |
| Mokola   | 22.44        | 2.69         | 10.45        | 0            | 35.58         |
| Sabo     | 9.88         | 0            | 5.41         | 0            | 15.29         |
| Total    | 78           | 6.28         | 43.15        | 3.84         | 131.27        |
| % aflatoxin | 59.54    | 4.78         | 32.76        | 2.93         | 100.01        |

(b)

| Location | AFB1<sup>a</sup> | SE | AFB2<sup>a</sup> | SE | AFG1<sup>a</sup> | SE | AFG2<sup>a</sup> | SE |
|----------|------------------|----|-----------------|----|-----------------|----|-----------------|----|
| Suya spices | 230.65**        | 1.31| 5.40**         | 0.34| 100.03**       | 0.25| 2.39**         | 0.12|

* means $P < 0.05$, significant, and ** means $P < 0.01$, highly significant.

microscopic views) and this is in agreement with the findings of Fabian et al. [2]; Yasair and Williams [3]; Giese [5]; Nwaiwu and Imo [11]; Kumar et al. [12]; Jonathan et al. [6, 7, 13]; and olayiwola et al. [10] who isolated similar organisms from spices and some other street vended foods.

Mokola and Agbowo samples were found to be highly contaminated with fungal organisms. These fungi were found to be responsible for the depreciation of the food value of the samples collected as explained by Table 1, although the colour and aroma were unaffected for a period of time (over one month); that is, no sign of deterioration was noticed on the outside.

The aflatoxin analysis carried out on the samples revealed that all the samples contain varying amount of aflatoxins except the control, 33% of the samples contain aflatoxin B<sub>1</sub>, 67% of the samples have aflatoxin G<sub>1</sub> while 33% of the samples contain aflatoxin G<sub>2</sub>, and the control was found to be free of aflatoxins (the four types; B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>).

Aflatoxin B<sub>1</sub> was found in all the samples except the control (Figure 2), but at various concentrations; this can be attributed to the fact that when aflatoxin is produced by either A. flavus or A. parasiticus, aflatoxin B<sub>1</sub> is the first metabolite released before others (aflatoxins B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>) depending on the production rate [25]. To arrange samples based on the concentration of aflatoxin they contain (from the highest to the lowest), we have Agbowo; Mokola; Sango; and Sabo, respectively. Of all the samples, Agbowo and Mokola samples were found to contain lethal dosage as they contain dosage of aflatoxin B<sub>1</sub> which is beyond tolerable limit (the standard being 20 μg/kg for human consumption).

Aflatoxin B<sub>2</sub> was detected in only samples from Sango and Mokola but is below or within the confines of tolerable limit while aflatoxin G<sub>1</sub> is found in all the samples like aflatoxin B<sub>1</sub> but the concentrations in the individual sample are below the limit except the amount contained in Agbowo sample (18.63 μg/kg) which can be considered as close to the danger limit. Aflatoxin G<sub>2</sub> was found in Sango and Agbowo samples where the concentration is very low.

Table 2 shows the effect of the aflatoxin concentration on each of the food products, Aadun (cereal based product) and “Suya spices” (pepper based product), and this indicates that the aflatoxins have significant effect on the food products. The aflatoxin analysis of the samples revealed that they all contain aflatoxins in varying amount and there were no detectible aflatoxin contents found in the control. 59.54% of the aflatoxins are aflatoxin B<sub>1</sub> with highest recorded in
The results from this study can be linked to some factors as follows:

(a) The air flora of the location considering the possibility of spores being carried by dust as the two areas are usually busy especially the Agbowo and Mokola sample that was collected when the construction of the flyover bridge at Mokola was still on.

(b) Low sanitary precautions taken by the food handlers.

(c) The raw materials used in making the “Suya spices” which are majorly pepper which have not been well screened by plant breeders against aflatoxins (Aflasave).

Therefore, we recommend safe handling of the spice and proper hygienic measures especially in Agbowo and Mokola areas as aflatoxin concentrations above tolerant limit were detected in these locations. We also recommend proper storage system of the vended spice as the mold spores are abundant in the air. Continuous check for aflatoxin detection on food materials should be encouraged as this may serve as checkmating of the safety of consumed food.

### Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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