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Fungal Contaminants Associated with *Vigna subtarranea* Cakes *(Okpa)* Hawked in Three Packaging Materials within Selected Areas of Makurdi, Benue State, Nigeria

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Abstract

Fungal contaminants and safety of ready-to-eat *V. subterranea* cakes *(Okpa)* packaged in milk tins, polythene, and banana leaves, hawked within 4 areas in Makurdi; Wurukum, Wadata, Modern Market and North Bank were assessed. Sixty *Okpa* samples were purchased from 5 sites at the selected locations, aseptically serially diluted in saline, cultured on Sabouraud Dextrose Agar, and incubated at 25°C for 7 days. Fungal growths were identified by gross colonial morphology, and microscopically, after staining with lactophenol cotton blue. Chi-square (χ^2) tested relationships between variables. P values ≤ 0.05 were considered significant. Five fungal species were isolated from all samples, irrespective of packaging material or location. They included *A. flavus*, *A. niger, Candida* sp., *Penicillium* sp., and *Rhizopus* sp. Across the 5 sites, *A. niger*, 151 (36.21%), was most isolated, while *Penicillium* sp., 52 (12. 47%) was least isolated. *Okpa* in tins had the most isolates, 144 (34.5%), while *Okpa* in banana leaves had the least, 132 (31.7%). The difference in Isolates from packaging materials was statistically significant *(p<0.05)*. *Okpa* from Benue Links Park had the highest isolates, 95 (27.8%), while the least isolates were from *Okpa* from North Bank Market; 75 (18.0%). The difference in fungal occurrence between sample locations was statistically significant *(p<0.05)*. *Okpa* hawked within selected areas of Makurdi were highly contaminated with fungi of public health concern. Setting standards, regular inspection by public health authorities, and educating vendors and patrons of *Okpa* on the importance of food hygiene and contamination prevention measures will help in safeguarding public health.

Keywords: Contaminants, Fungal, Makurdi, *Okpa*, Packaging, Ready-to-eat, Bambara nuts

Introduction

Ready-to-eat foods are products for immediate consumption that may require minimal further preparation, like warming, but not additional cooking [1]. Such foods include pastries, sausage rolls, pies, *moin-moin*, and burgers, etc. In many parts of Nigeria, ready-to-eat foods are commonly sold in several locations within the cities, towns, and villages [2]. Food-borne diseases related to the microbiological quality/safety of food are a public health concern and constitute major health challenges globally, which can predispose poor health conditions and affect socioeconomic development [3].

Okpa is a food prepared from the Bambara (*Vigna subterranea*) nuts, a seed crop of African origin popular because it thrives on soils infertile for other crops, such as beans and peanuts [4].

Bambara nut is commonly called *Okpa* by the Igbos, *Epa-roro* by the Yorubas, and *Kwaruru* by the Hausas. In the Tiv language, it is called *Akpukpa u Igbough*, and as *Okpa* by the Idoma and Igede of Benue State [4].

Okpa is nutrient rich. [5] showed that Bambara nuts are 19% protein, 63% carbohydrate, and 6.5% oils. Foods made from these nuts are therefore a good growth medium for microorganisms, some of potential public health importance [6].

Microorganisms such as molds, yeast, and bacteria are responsible for food spoilage and contamination. In various parts of Nigeria, several foods sold by vendors at bus stops, markets, highways, towns, and remote communities are reported to be heavily contaminated with bacteria and fungi [7].

While hunger satiation and nutritional needs are met through fast food, it is also necessary to ensure that it is safe from microorganisms and other contaminants [8]. There are several reports on the microbial loads of *Okpa* and other ready-to-eat foods in various parts of Nigeria [6]. However, because there is limited data on the fungal

safety status of *Okpa* sold in Makurdi metropolis, this study will help close this information gap.

Materials and Methods

Study Area

The study area was Makurdi, Benue State. The study was carried out at 4 locations within Makurdi: North-Bank, Wurukum, Wadata, and Modern Market (Fig. 1). Makurdi metropolis lies between latitudes 7˚33' 00" N and 7˚47' 00" N, and longitudes 8˚27' 00" E and 8˚4'00" E in Central Nigeria. It covers a land mass of 804 km^2 in a 16 km radius, with a population of 391,924 [9, 10].

Climatically, Makurdi is tropical and sub-humid, with two distinct seasons: the wet, from April to October, and the dry from November to March. Rainfall has an annual mean of 1190 mm. Mean annual temperature is 20.8 °C to 22.8°C, while average Relative Humidity (RH) is between 43% in January to 81% between July - August [11].

Makurdi is within the Guinea Savannah belt of Nigeria, where vegetation is characterized by tall grasses and trees of average height [9].

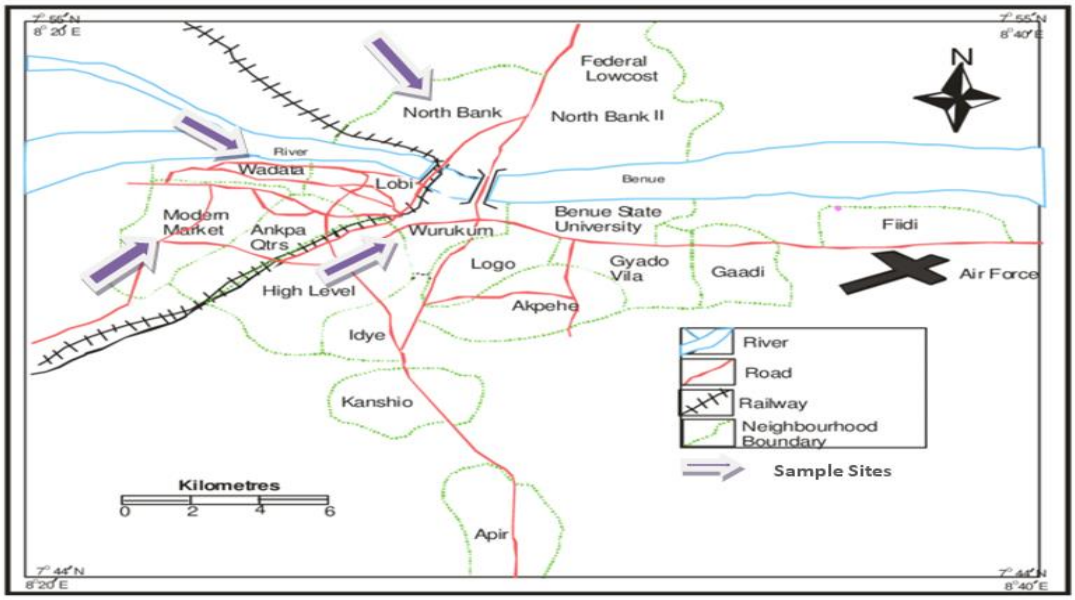

Figure 1: Makurdi Showing Study Locations *(Arrowed)* [12].

Collection of Samples

A total of sixty (60) samples were collected. Twenty (20) samples each packaged with three different materials: polythene, Banana leaves, and tins, purchased from five points of sale in Makurdi metropolis: Benue Links Park (Wurukum), NURTW Motor Park (Wurukum), Wadata Market, Modern Market, and North-Bank market.

Preparation of Samples

One (1) gram each of *Okpa* was aseptically transferred into a sterile beaker containing 9 mL of sterile normal saline and allowed to stand for 30 minutes, during which the beaker was repeatedly shaken to facilitate detachment of adhered microbes. The resulting supernatants were used for serial dilution.

Preparation of Media

Sabouraud Dextrose Agar (SDA) was prepared according to the manufacturer's instructions; the quantity of the dehydrated media to be used was weighed out and dissolved in the appropriate volume of sterile distilled water. The suspension was heated to properly dissolve and autoclaved at 121°C for 15 minutes.

Culture and identification of fungi

The tenfold serial dilution technique and pour plate inoculation, as described by **[13],** were used to culture fungi from samples. One milliliter of each supernatant was serially diluted. A tenth (0.1 ml) was taken from the 10^{-4} tube and aseptically transferred into a Petri dish containing SDA, using a sterile L-shaped glass rod to spread evenly on the medium. The plates were left undisturbed until solidified, then inverted and incubated at 25 °C for 7 days.

Gross colonial morphology was observed. Using two sterile needles, the fungal hyphae were teased into smaller pieces and emulsified in a drop of 70% alcohol on a clean glass slide. A drop of lactophenol cotton blue was added to the smear, covered with a cover slip, and viewed under 10x and 40x objective lenses. The result was compared with a standard identification guide.

Statistical Analysis of Data

Data was analyzed using IBM SPSS (version 22.0) and presented as simple descriptive statistics. Variables were compared using Pearson's Chi-square (χ^2) test. Values of $p < 0.05$ were considered statistically significant.

Results

Table 1 shows the type and frequency of fungi isolated from Okpa samples within Makurdi. The most prevalent isolate was *A. niger*, 151 (36.2%), and the least, *Penicillium* sp., 52 (12.5%). Other isolates were *A. flavus*, 98 (23.5%),

Rhizopus sp., 62 (14. 9%), and *Candida* sp. 53 (12.95%). Chi-square (χ^2) analysis showed a significant difference between the frequency of isolation of different species *($p < 0.05$)*.

Table 1: Frequency of Occurrence of Fungi Isolated from *Okpa* Samples

S/no.	Fungal Isolates	Frequency No. (%)
1.	*Aspergillus niger*	151 (36.21)
2.	*Aspergillus flavus*	98 (23.50)
3.	*Rhizopus* sp.	62 (14.87)
4.	*Candida* sp.	54 (12.95)
5.	*Penicillium* sp.	52 (12.47)
	Total	**417 (100)**

χ^2 =19.644; df=4; p = 0.001

Fungal isolates from Okpa samples in Relation to Packaging materials

Fungal species were isolated from *Okpa* in all the 3 packaging materials. The highest fungal occurrence was observed in *Okpa* samples in tins, 144 (34.53%). This was followed by those in polythene, 141 (33.81%). Banana leaves had the least occurrence of 132 (31.65%). A relationship was found between fungal occurrence and packaging materials *($\chi^2 = 15.839$; df = 2, p = 0.045)* (Table 2).

Table 2: Fungi Isolated from *Okpa* in Three Packaging Materials

S/no.	Packaging Material	Isolated Fungi					Total No. (%)
		Aspergillus niger No. (%)	*Aspergillus flavus* No. (%)	*Penicillium* sp. No. (%)	*Candida* sp. No. (%)	*Rhizopus* sp. No. (%)	
1.	Tins	59(39.1)	41(41.8)	10(19.2)	12(22.2)	22(35.5)	**144**(34.5)
2.	Polythene	45(29.8)	31(31.6)	20(38.5)	26(48.1)	19(30.6)	**141**(33.8)
3.	Banana Leaves	47(31.12)	26(26.5)	22(42.3)	16(29.6)	21(33.9)	**132**(31.7)
	Total	**151** (36.2)	**98** (23.5)	**52** (12.5)	**54** (13.0)	**62** (12.0)	**417** (100)

$\chi^2 = 15.839$; df = 2, p = 0.045

Distribution of fungal isolates by sampling location

Table 3 shows the distribution of fungal isolates with respect to location. The result showed the highest fungal occurrence of 95 (27.80%) was in *Okpa* samples from Benue Links, followed by NURTW Wurukum, 84 (20.10%), Wadata Market, 82 (19.70%), and Modern Market, 81 (19.40%). The least was North Bank with 75 (18.0%). A significant relationship between fungal occurrence and location was observed *($\chi^2 = 36.716$; df = 4, p = 0.002)*.

Table 3: Fungal Isolates from Sample Locations

S/no.	Sampling Locations	Isolated Fungi					Total No. (%)
		Aspergillus niger No. (%)	*Aspergillus flavus* No. (%)	*Penicillium* sp. No. (%)	*Candida* sp. No. (%)	*Rhizopus* sp. No. (%)	
1.	Benue Links Park	39 (25.8)	22 (22.4)	12 (23.1)	5 (9.3)	17 (27.4)	**95** (27.8)
2.	NURTW Wurukum Park	31 (20.5)	18 (18.4)	11 (21.2)	10 (18.5)	14 (22.6)	**84** (20.1)
3.	Wadata Market	27 (17.9)	11 (11.2)	15 (28.8)	16 (29.6)	13 (21.0)	**82** (19.7)
4.	Modern Market	28 (18.5)	23 (23.5)	6 (11.5)	17 (31.5)	7 (11.3)	**81** (19.4)
5.	North Bank Market	26 (17.2)	24 (24.5)	8 (15.4)	6 (11.1)	11 (17.7)	**75** (18.0)
	Total	**151** (36.2)	**98** (23.5)	**52** (12.5)	**54** (13.0)	**62** (14.9)	**417** (100)

$\chi^2 = 36.716$; df = 4; p = 0.002

Discussion

Fungi isolated during this study included *A. niger*, *A. flavus*, species of *Rhizopus*, *Candida,* and *Penicillium*. [14] studied the occurrence of fungal species in various ready-to-eat foods, and found that *A. niger* and *A. flavus*, as also found in this study, were among the most isolated fungi. The study also highlighted the significant presence of *Rhizopus, Candida*, and *Penicillium* species, indicating widespread fungal contamination of such foods.

A study by [15] on fungal contamination of street-vended foods found a high prevalence of *Aspergillus* and *Penicillium* species, consistent with findings of this study. The study also documented the significant presence of *Rhizopus*, *Candida*, and *Penicillium* species, indicative of widespread fungal contamination, and emphasized the role of environmental conditions and poor hygiene practices in contributing to fungal contamination. [4] also had similar findings.

The fungal species isolated in this study have been implicated in several human infections. *Candida* species, such as *C. albicafns*, *C. dubliniensis*, *C. parapsilosis, C. pulcherrina, C. tropicalis,* and *C. utilis,* are opportunistic pathogens of several infections, like oral cavities, oral thrush, gastroenteritis, or diarrhea when consumed in foods [16].

Penicillium species have been implicated in human infections. When ingested, they are toxigenic, cause allergic reactions, or respiratory issues [17]. *Rhizopus* sp. are implicated in mucormycosis, a potentially fatal disease of the brain, lungs, and sinuses, especially in the immunocompromised individuals [18].

In this study, *Okpa* in tins had the highest fungal occurrence, followed by Okpa packaged with polythene and banana leaves. A study by [19] found that fungal contamination was most prevalent in foods stored in tins. They found *Aspergillus* and *Candida* to be the most common isolated species. In this study, *Aspergillus* sp. was also the most isolated, followed by *Rhizopus* sp., while *Penicillium* sp. were the least isolated. This could be due to differences in fungi prevalent in the different study locations.

Another study by [20] on the microbial load of foods stored in various packaging materials found that tins provided a conducive environment for fungal growth. The study observed similar fungal species, such as *A. niger* and *Candida*, in tin-packaged foods, as also found in this study. [21] also analyzed ready-to-eat street foods packaged in different materials for microbial loads. Findings indicated that foods packaged in tins had the highest microbial counts, followed by polythene and banana leaves. The authors attributed the findings to the propensity of tins to develop rust and corrosion over time that could compromise the food's safety.

However, while rust or corrosion does not directly influence fungal growth, rust creates pits and crevices in containers that trap moisture and food particles, which can enhance fungal colonization and growth. Moreover, iron III oxide (Fe_2O_3) and other ions from rust can leach into foods and provide micronutrients, enhancing fungal growth [22, 23, 24].

Typically, vendors of *Okpa* use discarded milk tins that are prone to rust and corrosion. Such tins are also reused multiple times. Ideal conditions for fungal colonization and growth outlined above may have developed in them, leading to the high fungal contamination rates recorded. *Okpa* in tins is hawked without lids or covers, making them prone to seeding and contamination by fungal spores.

The finding that *Okpa* in polythene had the second highest incidence of fungi is an interesting observation, as it would be expected that polythene would be impervious to microorganisms. However, during the cooking, handling, and packing processes, polythene used for packaging may have developed nicks and cuts, some microscopic, which nevertheless, admitted spores of the isolated fungi.

Okpa packaged in banana leaves had the least prevalence of fungi. Unlike tins and polythene, banana leaves may contain microbial-inhibiting substances inherent in them [25, 26, 27]. These substances may have leached into the *Okpa* during the preparation and resulted in lower counts from the banana leaves packaged *Okpa*.

Conclusion

The microbial safety of ready-to-eat *Okpa* sold in Makurdi was assessed. The study found the presence of various fungal pathogens, with species such as *A. niger*, *A. flavus*, *Rhizopus*, *Candida*, and *Penicillium* prevalent. The highest occurrence found in tin-packaged *Okpa* underscores the role of environmental conditions and packaging materials in influencing microbial presence. The study recommends improved hygiene practices and careful consideration in the choice of packaging materials to ensure the safety of ready-to-eat *Okpa*.

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