Nature of Packaging Material and Bacteria Isolated from Ready-to-Eat *Voandzeia subterranea* (*Okpa*) Cakes Sold in Makurdi Metropolis, Benue State, Nigeria

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ABSTRACT

This study assessed the bacteriological safety of Okpa, a popular street food made from Bambara (Voandzeia subterranea) nuts. It determined the bacterial loads associated with the kind of materials used locally in packaging. Sixty (60) samples of Okpa, hawked in locally used packaging materials: polythene, banana leaves and open-ended tins, were investigated across four locations and sites popular with vendors. Bacteria were isolated from Okpa in all the packaging materials at a total frequency of 162 (100%), and included E. coli, Klebsiella spp., Salmonella spp. S. aureus and coagulase negative Staphylococci. E. coli was the most prevalent bacterium isolated 52 (32.1%). A significant statistical difference was found between the frequency of isolates ($\square^2$ = 25.7; p = 0.000). Samples at Modern Market and North Bank had the most bacterial isolates at 35 (21.6%) frequency each respectively. However, no statistical significance was found in the frequency of bacterial between sample sites ($\square^2$ = 144; p = 0.825). The highest bacterial load was found in Okpa sold in tins with 57 (35.2%) isolates. This was followed by Okpa in banana leaves with 53 (32.7%) isolates. No significant relationship was found in the frequency of isolates from different packaging materials ($\square^2$ = 9.816; p = 0.45). Isolates were mostly members of the enterobacteria and pathogenic in nature. These bacteria have been implicated in gastritis, typhoid and other infections of the digestive system. The opportunistic pathogen, S. aureus, was also isolated. The presence of these pathogenic bacteria suggests that Okpa sold in Makurdi poses a public health risk and may contribute to food-borne infections. Okpa sold in Makurdi should preferably be packaged in the more natural banana leaves. Though polythene had the least bacterial counts, other health concerns exist over the safety and use of polythene in foods. Okpa in tins should be avoided, as this container is not sealed and open. During hawking, the Okpa is exposed to the environment, which enhances contamination by bacteria. Education of both vendors and consumers on safety standards could help in protecting the public from hazards posed by consumption of unhygienic Okpa.

Keywords: Okpa, Packaging, Bambara nuts, Makurdi, ready-to-eat, Voandzeia subterranea

INTRODUCTION

Street foods have become popular, not only as a source of convenient and affordable food, but also as a main source of livelihood [1]. People who use such foods are often more interested in convenience rather than safety, quality or hygiene [2]. However, street food vendors often overlook the importance of the safety and nutritional quality of the food they serve, which can cause food-related diseases, even poisoning and death to customers [1]. Though the nutritional and hunger of consumers may be satiated via the consumption of street food, it is also necessary to ensure its safety from contaminants and microorganisms [3].

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Ready-to-eat foods are food products for immediate consumption or that may require minimal preparation like warming, but usually not additional cooking [4]. Ready-to-eat foods may be refrigerated, have reasonable shelf stability, require minimal heating or served cold. They include pastries, sausage rolls, meat pies, moin-moin, burger, and Okpa among others. They should not be contaminated, nor opportunities created for the emergence of bacteria after preparation of the food [5].

Okpa is a local fast food prepared from Bambara nut (V. subterranea). It is rich in nutrients that may also enhance and support bacteria, some, of public health significance, which contribute to spoilage and deterioration, making it unfit for consumption [6].

In Nigeria, a number of foods sold by food vendors operating in bus stops, markets, highways, towns and remote communities have been reported to be heavily contaminated with microorganisms [7].

There are several reports on the microbial load of ready-to-eat Okpa and other foods in various parts of Nigeria [7]. However, there is limited information on the bacteriological and safety status of Okpa sold in Makurdi metropolis.

Packaging materials are essential components used to enclose, protect, and preserve products during storage, transportation, and distribution. These materials serve a variety of functions, including safeguarding products from external influences, maintaining product quality, and providing information to consumers [8]. The choice of packaging material depends on factors such as the type of product, its intended use, environmental considerations, and regulatory requirements. Packaging materials play a crucial role in determining the microbial load and safety of food products.

Packaging materials should provide sufficient protection against physical damage during transportation and handling.

MATERIALS AND METHODS

Study Area

The study was conducted in Makurdi Metropolis in North-Central Nigeria, latitude 7˚33" N to 7˚47" N and longitude 8˚27" E to 8˚4" E [9]. Makurdi is tropical, sub humid, with two distinct seasons: wet and dry. Rainfall averages between 775 mm to 1792 mm, with a mean of 1190 mm. The mean annual temperature range is 20.8^{o}C to 22.8^{o}C, while the mean monthly Relative Humidity (RH) varies between 43% in January to 81% between July - August [10]. Makurdi has a population of 391,924 and is inhabited by diverse ethnic groups [11].

Samples Collection

A total of sixty (60) samples were collected for the study. Twenty (20) samples each packaged with three different materials evaluated, which are traditionally used for packaging Okpa; polythene, banana leaves and Tins respectively were purchased from five sales points within Makurdi metropolis. These were Benue Links Park, (BLP) NURTW Wurukum Park, Wadata Market, Makurdi Modern Market and North-Bank Market.

Sample Preparation

One gram (1g) of Okpa each was aseptically transferred into a sterile beaker containing 10 ml of sterile, normal saline and allowed to stand for 30 minutes. During this period, the Okpa gradually dissolved. The beaker was repeatedly shaken to facilitate detachment of adhered bacteria and ensure their even distribution. Subsequently, the resulting liquids were serially diluted.

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Media Preparation

Culture media were prepared according to the manufacturers' instructions. Suspended powder was brought to boil and sterilized in an autoclave at 121^{o}C/15 minutes. Xylose-Lysine Deoxycholate Agar was sterilized by boiling with frequent agitation.

Determination of Bacterial Loads

Ten-fold serial dilution techniques and pour plate method of inoculation as described by Cheesbrough [12] were employed to determine the bacterial loads of the samples. One milliliter (1ml) of each sample was serially diluted, and a tenth (0.1ml) was taken from the tube 10^{-4} and aseptically transferred into a Petri dish and covered with prepared and cooled molten nutrient agar medium. The plates were left undisturbed for about 5 minutes for proper solidification, they were then inverted and incubated at 37^{o}C for 24hrs. The colonies obtained after 24hrs of incubation, were counted using colony counter and recorded as colony forming units per milliliter (CFU/ml).

Isolation Techniques

The broth culture obtained from the samples, were sub-cultured repeatedly to obtain pure isolates. The growth characteristics of the isolates on each growth medium were observed and recorded.

Identification of Bacterial Isolates

This was as described by Cheesbrough [12]. Gram staining reactions, coagulase, catalase, sugar fermentation, citrate utilization, urease and indole tests were utilized for identification isolates.

Data Analysis

Data was analyzed using IBM SPSS statistical software, version 22.0 (IBM Corporation). Categorical variables were compared using Pearson's χ^2 test. At 95% confidence interval, p -values less than 0.05 ($p<0.05$) were considered statistically significant.

RESULTS

The bacteria isolated from ready-to-eat Okpa were identified as Escherichia coli, Salmonella spp., Staphylococcus aureus, coagulase negative Staphylococcus spp. and Klebsiella spp. (Table 1).

Table 1: Characteristics of Isolated Bacteria

Gram RXN	Biochemical Test												**Organism**
	Butt	**Cat**	**Cit**	**Coa**	**Gas**	**Glu**	**H_2S**	**Ind**	**Lac**	**Slope**	**Suc**	**Urea**	
-	A	+	-	-	+	+	-	+	+	A	+	-	E. coli
-	A	+	+	-	+	+	-	-	+	A	+	+	Klebsiella spp.
-	A	+	-	-	-	+	+	-	-	K	-	-	Salmonella spp.
+	A	+	+	+	-	+	-	-	+	A	+	+	S. aureus
+	A	+	+	-	-	+	-	-	+	A	+	+	CoNS

Key: Gram Rxn = Grain stain reaction, Ind = indole, Cit = citrate, Cat = catalase, Coa = coagulase, Lac = lactose, Glu = Glucose, Suc = Sucrose, Urea = urease, H_2S = Hydrogen sulphide, Gas = Gas Production, CoNS = Coagulase Negative Staphylococci, A = Acid, K = Alkaline, Butt = TSI agar slant, + = Positive reaction, - = negative reaction

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Bacteria Identified from Ready-to-eat V. subterranea (Okpa) Cakes within Makurdi Metropolis

Table 2 shows the frequency of isolated Bacteria from ready-to-eat Okpa. E. coli was the most frequently occurring bacterium, at 52 (32.10%). This was followed by Salmonella spp. 49 (30.24%), S. aureus 44 (27.16%), coagulase negative Staphylococcus spp. 14 (8.64%), and Klebsiella spp. 3 (1.85%). A significant difference between bacterial occurrences was observed (P = 0.000).

Frequency of Isolated Bacteria

Bacterial Isolate	**Frequency**	(%)
E. coli	52	(32.10)
Salmonella spp.	49	(30.24)
S. aureus	44	(27.10)
CoNS Staphylococcus spp.	14	(8.50)
Klebsiella spp.	3	(1.80)
Total	**162**	**(100)**

χ^2 = 25.7; df = 4; P = 0.000; CoNS = Coagulase negative Staphylococcus

Frequency of bacterial isolates by location

The Okpa samples from the North-Bank and Modern Markets respectively had the highest bacterial occurrence of 35 (21.60%), followed by samples from Benue Links 34 (21.1%), NURTW Wurukum 33 (20.40%), Modern Market 32 (19.80%) and Wadata Market 28 (17.30%). No significant relationship was observed in relation to the location (P = 0.825) (Table 3).

Table 3: Frequency of Bacteria Isolated at Sampling Sites/Locations

	Bacterial Isolate					
Location	**E. coli** (%)	**S. aureus** (%)	**Salmonella** spp. (%)	**CoNS Staphylococcus** spp. (%)	**Klebsiella** spp. (%)	**Total** (%)
Benue Links Park	11 (21.2)	8 (18.2)	10 (47.5)	4 (28.6)	1 (33.5)	34 (21.1)
NURTW Wurukum Park	11 (21.2)	11 (25.0)	9 (29.9)	1 (7.1)	1 (33.3)	33 (20.4)
Wadata Market	7 (13.5)	8 (18.2)	11 (45.3)	2 (14.3)	0	28 (17.3)
Modern Market	12 (21.2)	11 (25.0)	11 (45.3)	1 (7.1)	1 (33.3)	35 (21.6)
North-Bank Market	11 (21.2)	11 (25.0)	11 (45.3)	1 (7.1)	1 (33.3)	35 (21.6)
Total (%)	52 (32.1)	44 (27.2)	49 (30.2)	14 (8.6)	3 (1.9)	162 (100)

χ^2 =144.112, df = 4; P = 0.825; CoNS = Coagulase negative Staphylococcus

Frequency of Bacteria Isolated From Respective Packaging Materials

Table 4 shows the bacterial isolates by the kind of packaging material used. The highest bacterial occurrence was observed in samples in Tins 57(35.20%) followed by Banana leaves 53 (32.70%) and polythene 52 (32.10%). No significant relationship with packaging materials was, however, observed (p = 0.457).

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Table 4: Frequency of Bacterial Isolates with Respect to Packaging Materials Isolates

	Isolate					
Packaging Material	**E. coli** (%)	**S. aureus** (%)	**Salmonella** spp. (%)	**CoNS Staphylococcus** spp. (%)	**Klebsiella** spp. (%)	**Total** (%)
Polythene	15 (28.8)	13 (29.5)	18 (64.8)	6 (42.9)	0	52 (32.1)
Banana Leaves	18 (34.6)	15 (34.1)	13 (55.8)	4 (28.6)	3 (1.9)	53 (32.7)
Tins	19 (36.5)	16 (36.4)	18 (79.8)	4 (28.6)	0	57 (35.2)
Total	52 (32.1)	44 (27.2)	49 (30.2)	14 (8.6)	3 (1.9)	162 (100)

$\chi^2 = 9.816$; df = 2; P = 0.457; CoNS = Coagulase negative Staphylococcus

DISCUSSION

Most bacteria isolated in the study were pathogens, especially of the gastro-intestinal tract. The presence of E. coli, Salmonella and Klebsiella suggested that the sampled and tested Okpa may have become contaminated with fecal material of either human or animal origins. This probably would have been during the hawking process, as the temperature which the Okpa were subjected to during preparation would have been enough to kill off indigenous bacteria. The isolated organisms have been implicated in a number of human illnesses such as gastritis, diarrhea, typhoid fever, infections of the urinary tract, and continue to pose significant threat to the health of individuals particularly in a developing economy such as Makurdi's.

When commercial ready-to-eat foods were sampled for bacterial contamination, Chukwu et al. [13] also found *E. coli* as the most frequently isolated pathogen, followed by *Staphylococcus aureus* and *Salmonella* species, aligning with the findings of this study. They suggested the presence of *E. coli* as indicative of fecal contamination, often due to inadequate hygiene practices. In many parts of Nigeria, sanitary conditions, during food, especially commercial, preparation is poor. Such situations easily lead to contamination of such foods, thereby exposing patrons to pathogens and potential pathogens such as Staphylococcus spp. Statistically, the significant difference in the bacterial isolates has serious public health implications.

Another study by Adegoke and Komolafe [14] also found a high occurrence of *E. coli* in street-vended foods, highlighting it as a primary indicator of sanitation issues. The study noted that although *Klebsiella* species were present, they were less common, which is consistent with the findings of this research.

Though no significance was statistically found in bacterial loads in the packaging materials, Okpa packaged in tins had the highest bacterial loads, there were lower bacterial counts in banana leaves, while Okpa packaged in polythene had the least bacterial loads. Possible reasons for this observation could be that these packaging materials vary in their permeability to gases, including oxygen and carbon dioxide. Polythene, for example, may provide a barrier to oxygen, which probably inhibited the growth of aerobic microorganisms. Tins and banana leaves, on the other hand, may have allowed the entry of gases, thus affecting the microbial stability of the product [15].

Okpa, in tins, was exposed to the atmosphere, while Okpa in banana leaves, though completely wrapped, still admitted gases, which could have aided bacterial growth. Polythene packaging also probably created anaerobic conditions within the food, and so inhibited growth of isolated bacteria which were observed to be aerobic in nature. This could account for the lower bacterial loads in the polythene packaged Okpa. The completely sealed polythene may have promoted anaerobic conditions, unsuitable for the thriving and growth of bacteria isolated in this study. Moreover, bacteria from the environment could still have found entrance into the Okpa in tins and banana leaves, while polythene packaging would have made bacterial entrance into the packaged food more difficult.

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According to Okonko and Barber [16], packaging materials impact foods differently. They can provide insulation, helping to maintain the temperature of the product, crucial to the growth of pathogenic microorganisms. Certain packaging materials allow for the modification of the atmosphere within the package. How porous they are can influence the potential for microbial penetration, leading to contamination and spoilage.

The nature of the materials used could therefore have affected the amount of moisture available for bacterial activity and growth. Moisture is crucial because it profoundly affects the growth of microorganisms. High levels of moisture can promote the growth of bacteria, leading to spoilage and safety issues.

Out of the three materials evaluated, polythene would inhibit the entrance of moisture and better maintain the micro within the Okpa. Again, tins and banana leaves would have more readily admitted moisture from the environment and there-by made conditions more favourable to bacterial growth. However, unlike tins, banana leaves may contain bacterial inhibiting substances inherent in them, which may have resulted in lower counts from banana leaves packaged Okpa [17, 18].

Though the difference in the bacterial loads between sample sites was not statistically significant, the highest bacterial occurrence and loads were in samples from the North-Bank Market. This may be because the North-Bank area of Makurdi is particularly poor in environmental sanitation, with open defecation freely practiced. Potable and sanitary water is also scarce, so that water for domestic use is often sourced from untreated and unhygienic sources. These conditions could have invariably introduced high loads of bacteria into the Okpa from contaminated water, utensils and the environment.

Validating the above observation, according to Ukegbu et al. [19] and Odeyemi [20], packaged foods may be contaminated with microorganisms during the processing period. Sources of contaminants include the water for preparation, cooking utensils, the milking machine, food handlers and packaging materials, if not properly washed. The effect of these organisms not only reduces the nutritional value of the cake but also has serious health implications.

CONCLUSION

This study highlights concerns regarding the microbial safety of ready-to-eat V. subterranea cakes (Okpa) sold in Makurdi metropolis. The investigation revealed the presence of various bacterial pathogens, with *Escherichia coli* being the most common bacterium, indicating potential fecal contamination due to inadequate hygiene practices.

Investigating the impact of environmental factors, such as temperature and humidity, on microbial contamination can lead to more targeted strategies for significantly improving the safety of ready-to-eat foods like Okpa, reducing the risk of food borne illnesses and promoting better public health outcomes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Dexter, A. and Kemper, W. (2014). Two mechanisms for age-hardening of soil. Journal of Soil Science, 39: 163 - 175.
2. Mensah, P., Yeboah-Manu, D., Owusu-Darko, K. and Ablodey, A. (2002). Street foods in Accra, Ghana: How safe are they? Bulletin of World Health Organization. 80(7):546-554.
3. Monday, I. A, Oli, A.N, and Ikegbunam, M.N. (2014). Evaluation of the antibacterial activity of methanol and chloroform extracts of Alchornea cordifolia leaves. Journal of Scientific Research & Reports, 3(1):255-262.

4. Lucan, S.C., Maroko, A.R, Seitchik, J.L, Yoon, D., Sperry, L.E. and Schechter, C.B. (2018). Sources of Foods That Are Ready-to-Consume ('Grazing Environments') Versus Requiring Additional Preparation ('Grocery Environments'): Implications for Food-Environment Research and Community Health. Journal of Community Health. 43:886-895.
5. Alkerwi, A., Crichton, G.E. and Hébert, J.R. (2015). Consumption of ready-made meals and increased risk of obesity: findings from the Observation of Cardiovascular Risk Factors in Luxembourg (ORISCAV-LUX) study. British Journal of Nutrition. 28(113): 270-277.
6. Oranusi, S. and Braide, W. (2012). A study of microbial safety of ready-to-eat foods vended on highways: Onitsha-Owerri, southeast Nigeria. International Research Journal of Microbiology 3: 66-71.
7. Clarence, S.Y., Obinna, C.N. and Shalom, N.C. (2009). Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin City metropolis, Nigeria, African. Journal of Microbiology. Reserve 3: 390 - 395.
8. Rolain, N. (2013). Microbial quality and Safety of ready to-serve street foods vended in Allahabad city India. Internet Journal of Food Safety. 13:6-10.
9. Ologunorisa, E. T. and Tersoo, T. (2022). The Changing Rainfall Pattern and its Implication for flood Frequency in Makurdi, Northern Nigeria. Journal of applied Science and Environmental Management, 1(2):12-15.
10. NiMET (Nigerian Meteorological Agency) (2015). Climate of Makurdi Benue State. https://nimet.gov.ng
11. Tona, R. E. and Macus, I. K. (2022). Social Impact and People's Perception of Flooding in Makurdi Town, Nigeria. Special Publication of the Nigerian Association of Hydrological Sciences.
12. Cheesbrough, M. (2009). District Laboratory Practice in Tropical Countries Part 2. Cambridge University Press, UK. p. 434.
13. Chukwu, E. E., Odo, M. O. and Nwosu, I. S. (2020). Bacterial Contamination of Ready-to-Eat Foods in Urban Nigeria. African Journal of Microbiology Research 14(4): 245 - 255.
14. Adegoke, A. O. and Komolafe, K. A. (2019). Bacterial Contamination in Street-Vended Foods: A Study in Food Safety. Journal of Food Safety 38(2): 456 - 467.
15. Ghanem, M. Mitchell; A.L., Finn, R.D. and Gürel, F. (2013). Microbial composition of Kombucha determined by using amplicon sequencing and shotgun metagenomics. Journal of Food Science 85:455-464.
16. Okonko, N.J. and Barber, L.I. (2009). Assessment of Food Quality and the Associated Mycoflora of Okpa, a Local Recipe from Bambara Groundnut. Journal of Food Science. 4:1-5.
17. Ponmurugan, K. and Muhammed, M. (2013). Antibacterial and antioxidant activities of Musa specie leaf extract against multidrug resistant clinical pathogens causing nosocomial infections. Asian Pacific Journal of Tropical Biomedicals3:737-742.
18. Rita, W. S., Swantara, I. M. D., Asih, I. A. R. A. and Puspawati, N. M. (2020). Antibacterial activity and antioxidant capacity of selected local banana peel (Musa sp.) methanol extracts cultivated in Bali. International Journal of Agriculture, Environment and Bioresearch 5(03): 242–251.
19. Ukegbu, P.O., Uwaegbute, A.C. and Onyedinma, C.Q. (2015). Proximate composition of organoleptic evaluation of Bambara groundnut pudding (Vigna subterranea l thouars) produced by different methods. Journal of Sustainable Agriculture and the Environment. 1(16): 25-27.
20. Odeyemi, O. A. (2016). Public health implications of microbial food safety and foodborne diseases in developing countries. Food Nutrition Reserve. 60: 29819.