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Effects of packaging material on microbial load and safety of ready–to-eat *Voandzeia subterranean* cake (Okpa)

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Abstract

Background: Food-borne diseases remain a big challenge to food regulatory agencies. This study evaluated the microbiological safety of *Voandzeia subterranean* cake also called Bambara groundnut cake (BGC) packaged with cellophane and banana leaves.

Methodology: Twelve samples, 6 packaged in cellophane and 6 packaged in banana leaves were randomly selected from 6 strategic locations including, Government House Area (GHA), Eke- Awka market (EAM), UNIZIK junction (UZJ), Aroma junction (ARJ), and Nwagu junction, Agulu (NWJ). Two other samples were prepared fresh at School of Pharmacy, Agulu (SOP) which served as control. All samples were analyzed for total aerobic and fungi counts. Antimicrobial susceptibility evaluation was done on the isolates to determine their antibiogram.

Results: Microbial load of *V. subterranean* cake packaged in cellophane and banana leaves at 24 h post-preparation were $<10^4$ CFU.g^{-1} which is within safe limit of consumption. However, all samples packaged with banana leaves exhibited heavy contaminations with microbes at 48 h (above 10^5 CFU.g^{-1}). Conversely, there were no growths of fungi in samples packaged with cellophane. The organisms: *Micrococcus spp*, *Escherichia coli*, *Lactobacillus spp*, *S. aureus* and *Salmonella spp* were found as contaminant. All the bacterial isolates were resistant to Co-amoxiclav and Cloxacillin. *Salmonella spp* isolates were resistant to all the antibiotics tested. Only the *E. coli* isolates had a Multi-Antibiotic Resistance Index (MARI) of less than 30%.

Conclusion: The BGC packaged with cellophane are within the acceptable microbiological standard within the first day of production and compared better, microbiologically, than those packaged with banana leaves. However, the isolation

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of *E. coli* and *Salmonella spp* from the products questions the safety of the products and could spread Multi-Antibiotic Resistant isolates. From this study, good sanitary condition is recommended during preparation and packaging processes of *V. subterranean* cake with banana leaves.

Keywords: Bambara groundnut cake; Food safety; Microbial quality; Okpa; Ready-to-eat-food; *Voandzeia subterranean*

1. Introduction

Food-borne diseases constitute major health challenge, can predispose to poor health condition and affects socioeconomic developments in both developed and developing countries [1, 2]. Contamination of processed food during harvesting, preparation and packaging is the major cause of food borne diseases [3, 4]. Better consumer health is achieved through identification and control of sources of contamination. Studies have shown that microorganisms such as mold, yeast and bacteria are responsible for food spoilage and contamination [1, 5, 6]. Proper sanitary condition is therefore necessary in food preparation and packaging to avoid microbial contamination [7].

Ready-to-eat foods are foods or food products for immediate consumption or may require minimal preparation like warming but not any additional cooking [8]. Ready-to-eat foods may be stored in a refrigerator, have reasonable shelf-stabliliy, may need minimal heating or may be served hot [9]. They include: pastries, sausage rolls, meat pie, moin-moin, burger, okpa among others [10]. They should not be contaminated, nor opportunities created for emergence of bacteria after preparation of the food [11].

In various parts of Nigeria, a number of foods sold by food vendors operating in bus stop, markets, high way, towns and remote communities have been reported to be heavily contaminated with bacteria and fungi [12].

BGC is produced from *V. subterranean*, a plant belonging to the family of Fabaceae. *V. subterranean* is a legume that is indigenous to Africa and grows in areas with high and low rainfall. Its seed possess a similar shape as peanut [13]. Study by Okonkwo and Opara, [14] revealed that the seeds contain 19% protein, 63% carbohydrate and 6.5% oil and can be processed into flour. BGC is commonly called “Okpa” by the Igbo speaking populace, “Epa-roro” by the Yorubas and “Kwaruru” by the Hausas [12]. The cake is prepared by first removing the outer shell or coating of the seed, then milling into flour. The flour is then added some salts, spices like pepper, tumeric etc, sufficient water and palm oil or crude palm fruit extract before stirring or blending into a homogeneous paste. The paste is packaged with transparent polyethylene pack, banana leaves, tin or plastic containers before cooking to form the cake (BGC). The prepared cake is sold in Kiosks, open markets, garages or highway check points by vendors. It is mostly cherished by the Southern Eastern populace in Nigeria. It is commonly consumed at school by children and students as lunch. It is mostly served with cooled soft drinks or tea [15] or even water.

There are reports on the microbial load of several ready-to-eat Okpa and other foods in various parts of Nigeria [5, 12, 15]. However, there is limited information on the microbial safety status of Okpa sold in Anambra State of Nigeria. Also, a comparative study on the microbial quality of BGC packaged in cellophane and banana leaves is not available in this region. These knowledge gaps prompted this study.

2. Material and methods

2.1. Samples collection

A total of 12 samples of *V. subterranean* cake were used in the study. Two samples, one packaged with cellophane and another with banana leaves were purchased from five strategic points of sales including: Government House Area (GHA), Eke-Awka market (EAM), UNIZIK junction (UZJ), Aroma junction (ARJ), and Nwagu junction Agulu (NWJ). Two other samples were prepared fresh at School of Pharmacy, Agulu (SOP) which served as control. All freshly prepared samples were conveyed under sterile condition to the Pharmaceutical Microbiology and Biotechnology Laboratory, Faculty of Pharmacy, Agulu for microbiological analysis.

2.2. Samples processing and Isolates identification

A total of 12 samples of BGC were collected hot and allowed to cool in a sterile cupboard. Ten grams of each sample was emulsified using a surfactant (1% polysorbate 20) and made up to 100 mL. The emulsified samples were maintained at 40 ^{o}C for at least 30 mins before subjection to bioload determination using pour plate technique. Dilutions (10, 100 and 1000 - fold) were made with normal saline. Using pour plate technique, the total aerobic, enterobacteriaceae, and fungi

was assessed on Nutrient agar, MacConkey agar and Sabouraud Dextrose agar. Here, 1 mL of the diluted sample was transferred into a sterile petri dish and 19 ml of the molten agar was added, mixed thoroughly and then allowed to stand on the bench for 10 mins. Other selective media such as Mannitol salt, *Salmonella Shigellae*, Cetrimide and Methyl rugosa agars were also used to specifically detect other bacteria like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Lactobacillus species*.

Thereafter, the plates were incubated at their respective optimal temperatures (37 ^{o}C for 24 h for bacteria and 25 – 28 ^{o}C for 72 h for fungi). Colony forming unit per gram (CFU.g^{-1}) were counted using a colony counter. All the isolated microorganisms were identified through microscopic and specific confirmatory biochemical tests.

2.3. Antibiogram Study

Antibiotics susceptibility testing was carried out on the identified isolates using Agar diffusion methods on Mueller-Hinton Agar (MHA) for the bacteria isolates and Sabouraud Dextrose agar for the fungi isolates as described by Clinical Laboratory Institute Standard [16]. Commonly used antibiotic discs (Ofloxacin, Co-amoxiclav, Ceftazidime, Ceftriaxone, Gentamycin, Cefuroxime, Erythromycin and Cloxacillin, Oxoid UK) were used. Briefly, 0.5 McFarland suspensions of the isolates were inoculated onto the MHA and SDA media and allowed for to dry and diffuse for some 5 mins. Then the antibiotic discs were aseptically placed on the solidified agar. Finally, the plates were incubated appropriately.

The Inhibition zone diameter (IZD) observed after 24h incubation at 37 ^{o}C was measured to the nearest millimeter for each of the antibiotic tested against each isolate. The study classified the isolates as susceptible, intermediate or resistant according to interpretative chart of complete growth inhibition zone diameter sizes for bacteria with reference to [16]. Results were presented as average of duplicate experiments.

3. Results

Our results show that the microbial load of the aerobic organisms was below 10^4 at 24 h for both cellophane and banana packages (Table 1). However, at 48 h, the values were beyond 10^6 for both packaging materials. Student *t*-test showed that there is significantly lower (p value < 0.0001) microbial load with cellophane package than with banana package at 24 h and 48 h. From the results in Table 1, observed aerobic count in the BGC samples were within the acceptable level of < 10^3 CFU.g^{-1} (Class B) at 24 h suggesting that the consumption of the Okpa samples prepared with Cellophane and Banana leaves within 24 h did not pose deleterious effects.

Table 1 Mean aerobic count of *V. Suberrenea* cake samples

Sample		**Colony forming unit (CFU/10 g)**	
		24 hours	**48 hours**
Packaged with cellophane	GHA	10^3- <10^4	≥ 10^7
	ARJ	10^3- <10^4	≥ 10^7
	UZJ	10^3- <10^4	≥ 10^7
	EAM	10^3- <10^4	≥ 10^7
	NWJ	< 10^3	≥ 10^5
	SOP	10^3- <10^4	10^6-<10^7
Packaged with banana leaves	GHA	10^3- <10^4	≥ 10^7
	ARJ	10^3- <10^4	≥ 10^7
	UZJ	10^3- <10^4	≥ 10^7
	EAM	< 10^3	≥ 10^6
	NWJ	< 10^3	10^6-<10^7
	SOP	< 10^3	10^6-<10^7

There were no observable fungus counts at 24 h for both packaging materials and the control and at 48 h for cellophane packaging. However, at 48 h, there were counts beyond 10^3 for all the banana leave samples, except sample from SOP (Table 2).

Table 2 Total fungi count of *V. Suberrenea* cake samples

Sample		**Colony forming unit (CFU/10 g)**	
		24 h	**48 h**
Packaged with cellophane	GHA	-	-
	ARJ	-	-
	UZJ	-	-
	EAM	-	-
	NWJ	-	-
	SOP	-	-
Packaged with banana leaves	GHA	-	10^3- <10^4
	ARJ	-	10^3- <10^4
	UZJ	-	10^3- <10^4
	EAM	-	10^3- <10^4
	NWJ	-	10^3- <10^4
	SOP	-	-

- indicates absent

The organisms isolated were *Micrococcus spp, Escherichia coli, Lactobacillus spp, S. aureus* 1, *S. aureus* 2 and *Salmonella spp*. These organisms were mostly susceptible to Ofloxacin and Gentamycin. *Salmonella spp* was resistant to all the antibiotics tested. Also, all the isolates were resistant to Co-amoxiclav and Cloxacillin (Table 3). Only the Presence of *Micrococcus spp, Escherichia coli*, *Lactobacillus spp*, *S. aureus* and *Salmonella spp* in samples suggests poor hygienic and sanitary conditions employed by producers.

Table 3 Antibiotic susceptibility of the microbial isolates of the BGC

Organism isolated	**OFL**	**COAM**	**GAZ**	**CRX**	**GEN**	**GTR**	**ERY**	**CXC**	**MARI = a/b*100**
	Inhibition Zone Diameter (mm)								
Micrococcus spp	26	0	0	0	23	9	30	0	62.50
Escherichia coli	31	0	13	20	21	30	8	0	25.00
Lactobacillus spp	31	0	0	0	24	0	0	0	75.00
S. aureus 1	24	0	0	18	28	12	29	0	37.50
S. aureus 2	29	9	9	14	30	12	28	0	37.50
Salmonella spp	0	0	0	0	0	0	0	0	100
Number Resistant	1	6	5	3	1	3	3	6	

Note: a= Number of antibiotics organism is resistant to; b= Number of antibiotics tested (= 8); Resistance = 0-11; Intermediate = 12-18; Susceptible = ≥ 19; OFL (Ofloxacin), COAM (Co-amoxiclav), (GAZ) Ceftazidime, CRX (Ceftriaxone), GEN (Gentamycin), GTR (Cefuroxime), ERY (Erythromycin), CXC (Cloxacillin)

4. Discussion

Traditionally, BGCs are packaged in banana leaves. Recently, the emergence of same products packaged in cellophane materials flooded the markets. The present study revealed that poor hygienic nature of packaging materials used for

BGC could affect the microbial load. It was clearly observed that most samples packaged with nylon were free of fungal contaminants compared with samples packaged with banana leaves. Earlier studies had reported the presence of microbial contaminants in food products and medicines [17-20].

The observed aerobic count in the BGC samples within the acceptable level of < 10^3 CFU.g^{-1} (Class B) at 24 in table 1 suggests that the consumption of Okpa samples prepared with Cellophane and Banana leaves within 24 h, and did not pose deleterious effects. According to the Centre for food safety and environmental hygiene department [21], food samples with <10^4 CFU.g^{-1} are classified as satisfactory (Class A), those between 10^4 - <10^5 CFU.g^{-1} could be classified as acceptable (Class B), while those above ≥ 10^5 CFU.g^{-1} could be classified as Class C (unsatisfactory). Oranusi and Braide [12] also revealed that microbial contamination that is above 10^6 CFU.g^{-1} are unacceptable for consumption and could be risky due to its contribution to food borne disease.

The observed aerobic count above 10^5 at 48 h suggests that they were not within the acceptable level (they are in Class D), probably due to longer time of storage. Thus, it is safer to consume BGC prepared within 24 h.

Absence of fungi in samples packaged with cellophane for 48 h as against samples packaged with banana leaves (Table 2), suggests that polyethylene packaging provided better storage condition. Although, it has been reported that packaging with leaves provide good preservative condition due to their natural antimicrobial and antioxidant property [22], the observed result could be an indication of contamination of leaves during harvesting and handling during the packaging of BGC. Thus, those leaves could have been poorly washed or unwashed before use. According to Okeke *et al.,* [23], dried banana leaves used in wrapping Okpa could harbor microorganism whenever they are not properly washed before use. Also, some products that are left over are normally poorly re-heated for sales the following day, resulting to microbial contamination [23]. Absence of fungi in BGC prepared and packaged in School of Pharmacy, Agulu could be due to strict adherence to proper packaging in sanitary condition.

The organisms isolated were *Micrococcus spp, Escherichia coli, Lactobacillus spp, S. aureus* 1*, S. aureus* 2 and *Salmonella spp* . These organisms were mostly susceptible to Ofloxacin and Gentamycin. *Salmonella spp* was resistant to all the antibiotics tested. Also, all the isolates were resistant to Co-amoxiclav and Cloxacillin (Table 3). Only the multi-antibiotic resistance index (MARI) below 30% posed by *E. coli* isolates in Table 3 suggests a very high multi-drug resistance character of the isolates.

Presence of *Micrococcus spp, Escherichia coli*, *Lactobacillus spp*, *S. aureus* and *Salmonella spp* in samples suggests poor hygienic and sanitary conditions employed by producers. These organisms have been reported to be implicated in food borne diseases [24, 25]. For instance, *S. aureus* has the ability to survive in dry environment such as clothing, inanimate surface, hands, and skin for some period of time after initial contact. Reports have shown that ingestion of *S. aureus* contaminated food could be associated with vomiting, nausea, abdominal cramp and other complications [26].

Studies by Oranusi and Braid [12] revealed the presence of related organisms in ready-toeat foods, including Okpa sold by vendors along Onitsha-Owerri highway, South-East Nigeria. They recorded coliform counts above 10^4 for BGC, sliced Pineapple, Egg roll, Apple and Elulu-ngwo. Studies by other researchers likewise revealed presence of related micro-organisms in meat pie, sausages and sea foods [10, 27-29].

Although microorganism are ubiquitous in nature and have been found virtually in all ready-to-eat food at within a particular range, the level of contamination observed in BGC packaged with cellophane could have occurred through improperly washed hands, exposure of cellophane to air-borne pathogens, opening of cellophane by blowing air into them before the infilling with already prepared BGC in the liquid form.

Unlike the cellophane package, the leaves package can easily acquire microorganisms from the environments and the microbes so acquired find the environment more conducive for growth except if the leaves have good antimicrobial property [30]. The leaves packaging material tend to provide warm, moisturized and organic environment necessary for the microbial growth and multiplication.

The isolation of multi-drug resistant organisms from the BGC is a serious concern as it has been reported [31, 32] severally the drug resistant organisms can spread from food to human. The contamination of the BGCs with *E. coli* and *Salmonella spp* queries their safety for consumption and points to possible unhygienic procedure of processing them. It is recommended that aseptic methods should be followed during processing and that the products should be consumed while hot.

5. Conclusion

The study revealed that BGC packaged with cellophane and sold by food vendors in Awka environs are within the acceptable microbiological standard within the first day of production and they compared better, microbiologically, than those packaged with banana leaves. In light of the forgoing, it is necessary for producers of BGC to adopt good sanitary conditions. Use of antimicrobial treated packaging material as well as clean utensils and water during production are highly recommended.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest exists.

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