

Spore Forming Bacteria in Leftover Fried Foods Sold on Evening Roadside Table-Stands of Mbuoshimini Area of Port Harcourt

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ABSTRACT

The study screened for bacterial endospores in ready to eat fried foods sold in the evenings on roadside table-stands by food vendors in Mbuoshimini Area of Port Harcourt. Presence of bacteria endospores in fried foods, particular leftovers poses serious threat because the bacteria is resilient and can survive low frying temperatures. Fifteen (15) samples each of beans-paste (akara), fried potato and fried yam each were collected/purchased and taken to the laboratory for aerobic and anaerobic bacteria analysis. Result from the analysis showed a mean aerobic bacterium counts of 7×10^3 , 2×10^3 and 1×10^3 CFU/g for beans-paste (akara), fried potato and fried yam respectively. However, no anaerobic bacteria counts were noted as anaerobic colonial observation was visibly absent. A total of thirty-four (34) aerobic bacteria isolates were recovered, from which two (2) genera, *Bacillus* and *Proteus* were identified. All *Bacillus* identified, showed spores at the terminal ends of the rods on viewed under the microscope. However, *Proteus* Genius showed no spore formation. The implication thereof is that food vendors should beef up their heating or reheating control measures to at least 74°C to ensure non-survival of aerobic bacteria particularly, the *Bacillus* and eventually the safety and health of fried food consumers.

Keywords: Bacteria, Consumers, Endospore, Fried-foods, Food-Vendors, Leftover, Roadside, Table-stands.

Introduction

Some fried food such as yam and plantain are prone to post harvest contamination by diverse microbes (bacteria, fungi etc.) and these foods can endanger the public consumers by causing various foodborne diseases such as botulisms, gas gangrene and even fatal illnesses (Shah & Patel, 2022). At the instance of frying with the use of oil, it is supposedly believed that food contaminants are eliminated. However, bacterial endospore cannot be overemphasized/ considered (Pereira, 2022). According to Ahmed (2024) Bacteria endospore present in foods before and after frying remains a threat to the public consumers. Bacteria spores specifically, *Clostridium* sp. do have the capacity to survive high cooking temperature, hence pose health risk to consumers (Palacios & LeJeune, 2011).

Palacios and LeJeune (2011) stated that endospore forming bacteria are generally considered non-pathogenic, their presence can serve as an indicator of foodborne health risk, hence monitoring them can help food producers ensure that harmful pathogens are not present on the final products. The spores produced are therefore aimed at resisting the effect of external interference to the bacteria cell (Palacios & LeJeune, 2011). Spores are mostly produced in an adverse environmental condition, for the survival of the bacteria (Nicholson *et al.*, 2000). Adverse environmental condition such as heat, chemicals, radiation, etc. which ordinary would inhibit bacteria proliferation initiates the formation of spores for the bacteria survival and pathogenicity. The challenge thrown-off from spore forming bacteria cannot be over emphasized.

According to Anderson et al. (1995) the food industry is increasingly, confronted with resistant spore forming bacteria that may have gained entry into food via processing or packaging, following the highly increasing marketing of convenience food which are supposedly leftovers. According to Morrison (2025) the leftovers are surplus, been that they are not completely sold out, and the retailer cannot do away but re-fry, to make good for consumption. Consequently, leftover foods can be worrisome to retailers, were-in their capital or profits may be affected and for that retailers preserve them for later use, the following day (Morrison, 2025). The sale and consumption of fried foods such as yam, plantain, potato etc. in the evening have become increasingly common in the streets of Mgbuoshimini due to their affordability, accessibility, and convenience. However, endospore properties of bacteria continue to remain a public health challenge (Pereira, 2022). According to Pereira (2022), spores present in fried foods after preparations remain a threat to the public as some foods are regarded leftovers when retailers fail to sell them. Basically, spores forming *Clostridium* sp. have the capacity to survive high cooking temperature, hence pose a significant health risk to consumers of food associated with the bacteria (Yip, 2024). Following this and other challenges in the food industry, there is need to evaluate spores forming bacteria in suspected leftover fried food sold in the evenings along road sides on table-stands in parts of Mgbuoshimini, Port Harcourt. Consequently, knowledge on these challenges thrives to inform consumers' choice on purchase and hence increased awareness on safer purchase.

Materials and Methods

Study Area

The study area is Mgbuoshimini Community in Obio/Akpor Local Government Area (OBALGA) of Rivers State, Nigeria where the sale and consumption of fried foods in the evenings along the streets of Mgbuoshimini is a common practice. The Mgbuoshimini Community is bounded by several communities that make up the area namely; Rumuepirikom, Rumueme, Rumuolumeni amongst many others. The study area is made up of so many streets/roads where table market are visibly seen.

As a densely populated area, the streets have high population of human presence and vehicular movements, and being a host community to some oil servicing companies.

Collection of the Fried Food Samples

Samples for the study were collected from street table-stands vendors from selected spots in different locations. Fifteen (15) samples each of beans-paste (akara), fried sweet potato and fried yam each (making a total of 45 samples) were purchased and aseptically collected from street table-stand vendors in Mgbuoshimini.

The samples were thereafter immediately transported in ice-cool box to the laboratory of the Department of Microbiology, Faculty of Natural and Applied Sciences, Ignatius Ajuru University, Rumuolumeni Port Harcourt for aerobic and anaerobic bacteria investigation as well as spore forming property.

Preparation of Samples (Fried yam, potatoes and beans paste)

On arrival in the laboratory, each sample was aseptically cut into smaller bits with the aid of a sterile scalpel after which each sample was mashed in a sterile mortar with a pestle. One gram (1g) each of the meshed beans-paste (akara), fried sweet potato and fried yam samples were then added into a test tube and serially diluted into several fractions. The process was carried out as documented by Willey *et al.* (2008) and a 10^{-1} dilution was adopted for the investigation.

Preparation of Media

For aerobic bacteria determination, media preparation involved weighing some quantity of nutrient agar (3g) and dissolving them in distilled water (100ml), thereafter the composition was autoclaved as instructed by the manufacturer. For anaerobic bacteria determination, blood agar media was prepared and adopted.

Hence, some part of previously prepared nutrient agar media was allowed to cool before some quantity of sterile human blood (5ml) was added to the said nutrient media that is cooled at 50°C to obtain a blood agar medium (Ryan & Ray, 2004).

Enumeration of Total Aerobic Bacteria

Basically, nutrient agar medium was used to determine counts of aerobic bacteria, using spread plate count method. From the sample filtrate, 0.1ml aliquot was spread plated on the nutrient media, incubated at 37°C for 24 hours. Total bacteria counts were enumerated; that is, at the end of the incubation periods, colonies that developed were counted and recorded as colony forming unit per gram (CFU/g) of the food sample (Ryan & Ray, 2004).

Enumeration Total Anaerobic Bacteria

The blood agar medium was used to determine counts anaerobic bacteria were using spread plate method. From the 10⁻¹ diluted sample filtrate, 0.1ml aliquot was inoculated onto the blood agar media, incubated in the presence of carbon dioxide for 24 hours. Anaerobic bacteria were enumerated thereafter. That is, at the end of the incubation periods, colonies that developed were counted and recorded as CFU/g (Sapkota, 2022; Ryan & Ray, 2004).

Identification and Characterization of Bacteria Isolates

The isolates were characterized and identified based on their colonial morphological features such as; texture, colour, shape, size and elevation. Biochemical tests were also carried out for proper identification of the isolates. The test carried out are as follows; catalase, indole, methyl red, Voges -Proskauer, citrate utilization, urease and glucose test.

Glucose Test

The glucose test employed the use of glucose broth, which was prepared in the right proportion constituent. One-gram (1g) of glucose ingredient was added into eighty mill (80ml) of peptone water and stirred thoroughly to solubilize the sugar, thereafter twenty milliliter (20ml) of 0.2% (w/v) phenol red indicator was added to the solution, now referred to as glucose broth. Thereafter 10ml of the broth was dispensed into test tubes contained in it inverted Durham's tubes and the broth autoclaved at one hundred and twenty-one degrees centigrade (121°C) for fifteen (15) minutes. The sterile broth was allowed to cool, and the test isolate inoculated into it.

The broth was then incubated at thirty-seven degrees centigrade (37°C) for twenty (24) hours. An orange colour change of the medium signified a positive result for glucose while the absence signified no glucose utilization (Sapkota, 2022).

Catalase Test

The test involved the inoculation in drops, hydrogen peroxide on the test isolate, already impregnated on a clean microscopic slide. The procedure was done with the aid of a wire loop and the test isolate smeared evenly. Few seconds after the procedure, the slide was observed for bubbles. Catalase-positive bacteria were signified by the production of bubbles of oxygen (Sapkota, 2022).

Indole Test

The test bacteria were inoculated into a freshly prepared indole medium, a composition of nutrient broth, peptone water and tryptone broth with the aid of a sterile wire loop and thereafter, the medium incubated at thirty-seven degrees Celsius (37°C) for forty-eight (48) hours. After incubation, a red/pink layer formed on top of the broth indicated indole positive while a reverse of yellow indicated indole negative (Sapkota, 2022).

Methyl Red (MR) Test

The test involved the use of MR/VP agar, a glucose phosphate composition. The MR/VP agar was prepared into a broth and then sterilized, allowed to cool before the isolate was inoculated. Following the inoculation, the broth was incubated for four days at thirty-seven degrees Celsius (37°C). Thereafter 5 to 6 drops of methyl red reagent were added to the broth, stirred thoroughly, and allowed to stand for 5 minutes. The broth culture was then examined for color change (Sapkota, 2022). A colour change of the broth when compared to the control indicated MR positive while a non-colour change indicated an absence.

Voges Proskauer (VP) Test

The test involved the use of glucose phosphate constituent. The glucose phosphate constituent was prepared accordingly and dispensed into test tubes.

The broth was sterilized, allowed to cool before a test isolate was inoculated into it. Following this, the broth was incubated for four days at thirty-seven degrees Celsius (37°C). Thereafter, 1.5 ml of 5% alcoholic alpha naphthol and 0.5 ml of forty percent (40%) aqueous Potassium Hydroxide (KOH) was added and observed. A change in colour of the broth from pink, indicated positive VP, while a non-change of the broth indicates negative VP (Sapkota, 2022).

Citrate Utilization Test

This test involved the use of Simmons citrate agar, a composition of bromothymol blue as an indicator. Hence, the citrate agar was prepared and with the aid of a sterile wire-loop the isolate was inoculated on the citrate medium and the medium incubated at thirty-seven degrees Celsius (37°C) for twenty-four (24) hours. A citrate positive result was indicated by a change in colour of the indicator from green to blue (Sapkota, 2022).

Urease Test

The urease test involved the use of urease agar supplemented with phenol red indicator. In carrying out the urease test, the isolate was streaked on the surface of a freshly prepared urease agar media slant and the component incubated for forty-eight (48) hours at thirty-seven degrees centigrade (37°C). Following the incubation, the media was carefully examined for the development of a pink colour on the surface of the media. Thus, the development of bright pink colour indicated urease positive bacteria while the reverse, negative (Sapkota, 2022).

Motility Test

This test was used to determine the motility of the isolates. Motility of an organism is determined by the presence of flagella (a locomotory organelle). The motility test involved the use of nutrient agar media, were in the media was prepared to a semi solid state.

The semi solid media was prepared, dispensed into test tubes and the isolate picked with a sterile straight wire into it by stabbing. Thereafter, the medium in the tube was incubated at 37°C for 24 - 48 hrs. Growth in diffuse form from the line of stab into the medium indicated a positive result, whereas growth only along the line of stab indicated a negative result (Sapkota, 2022).

Spore Formation

The presence of bacteria spore was determined by carrying out staining and microscopic procedure. The test bacteria were inoculated on a clean microscopic glass slide, followed by staining with malacite green stain. Thereafter the slide was viewed under a light microscope at 100 objective lenses (Sapkota, 2022).

Results

The results of the enumeration of aerobic and anaerobic bacteria load from the fried food samples are presented in Table 1. It showed that the fried food samples under investigation had varied average loads of aerobic bacteria and an absence of anaerobic bacteria. For beans paste, an average aerobic count of 7.0×10^3 CFU /g was recorded while fried potato had 3.0×10^3 CFU /g counts. For fried yam investigation, an average aerobic count of 1.1×10^4 CFU /g was recovered.

The colonial classification of the aerobic bacteria isolates are as presented in Table 2, showed the colonial appearance of the aerobic isolates in terms of color, size, elevation, edge, opacity, shape and Gram's reaction. Isolates grouped (A) were milky in color, large sized convex entire edge that is opaque and round with a Gram-positive reaction. While isolates grouped (B) were identified white, small sized with low curved opaque rounded colonies that is Gram positive.

Table 1: Enumeration of Aerobic and Anaerobic Bacteria Load in the Fried Foods

Bacteria	Colony Forming Unit Per gram (CFU/g) of Fried Food Sample		
	Beans Paste	Potato	Yam
Aerobic Bacteria	7.0×10^3	3.0×10^3	1.1×10^4
Anaerobic Bacteria	0	0	0

Table 2: Colonial/Morphological Characteristics of the Isolated Aerobic Bacteria

Isolate	Color	Size	Elevation	Edge	Opacity	Shape	Gram Reaction
A	Milkish	large	Convex	Entire	Opaque	Round	+
B	White	Small	Low	Curve	Opaque	Rod	+

Key: Positive = +

Table 3 shows the biochemical screening results of the aerobic bacteria isolates as well as spore formation property, as they showed key confirmatory results. The biochemical result featured isolates *Bacillus* spp. and *Proteus* spp. Biochemical report for isolate A showed a swarming motion (motility positive) and a urease positive report. However, indole was negatively reported.

Isolate B showed strong catalase reaction, hence the isolates expressed vigorous bubbles, indicated *Bacillus* spp. with a follow up of glucose fermentative property. Table 3 further showed the spore formation property of the isolates isolated. Result indicated all Group B isolates, *Bacillus* spp. were able to express spore formation while all Group B isolates, *Proteus* sp. were unable to express spore features.

Table: 3 Biochemical Characteristics of the Isolated Bacteria

Biochemical Parameter									Probable organism
Motility	Urease	MR	Catalase	Citrate	Glucose	Indole	VP	Spore	
+	+	+	+	+	-	-	+	-	<i>Proteus</i> sp.
-	-	+	+	+	+	-	+	+	<i>Bacillus</i> spp.

Key: + = Positive, - = Negative, MR = Methyl Red, VP = Voges Proskauer.

Discussion

The difference in the counts of aerobic bacteria in the fried food samples reflects high storage concentration following report by Adedeji et al. (2013). Adedeji et al. (2013) stated that total plate count of fried food revealed fewer loads whereas on increased storage time, an increased load of total bacteria is accounted. The loads of aerobic bacteria so recorded fell below the recommended limit of 105 cfu/g for ready to consume food by international commission on microbiological specification for food (Adedeji et al., 2013). Ochei et al. (2014) noted that viable counts of aerobic bacteria in the fried food samples are also as a result of some cells that maybe viable but non-culturable. Hence, counts may differ in this case as observed in the bean cake, fried potato and fried yam. According to Baron et al. (2020) the presence of aerobic bacteria in the food samples may promote opportunistic infections however, aerobic bacteria have not been linked to pathogenic conditions (Hallt & Carroll 2016). Consequently, their presence as reported in several studies on food indicated insufficient sanitary condition.

This study which recorded zero concentration of anaerobic bacteria however if present may cause infection there-off (Bintsis, 2017). Anaerobic bacteria are a common cause of foodborne illness, and it can be found in various food samples due to several factors (Bintsis, 2017). According to Bintsis (2017) as insignificant reported, small concentrations of anaerobic bacteria have the ability to cause diseases in human and animals that come in contact with it. Morphological and biochemical identification of the isolates, *Proteus* spp. and *Bacillus* spp. were isolated and so identified, *Proteus* sp. and *Bacillus* sp. are normal flora of the gastro-intestinal tract. Similarly, according to Bankole et al. (2005) microorganisms have been pointed to be associated with the palms of food-vendors. The *Bacillus* sp. and *Proteus* sp. which were isolated as identified in the fried food may have gotten entry into the fried foods from the perch of house fly, or through droplet/dust around the table-stands (Ochei et al., 2014). Although, *Bacillus* spp. exists in the skin / hand of humans as a normal flora; hence may have been contact with the fried foods (Madueke et al., 2014).

The *Bacillus* sp. spore formation in the fried food serve as a pointed that denotes the *Bacillus* sp. survived frying even after precooked as alleged in this study. Consequently, according to WHO (2016) a number of observational studies showed that street foods are sometimes held at improper temperature. However, spore forming bacteria have been implicated in canned food that were processed and packaged in high pressured heat (Murray et al., 2013). Over the years, *Bacillus* spores have notably been identified in dishes cooked at usual frying temperature, although this study notably observed them in beans cake, fried yam and potatoes. This showed they have the ability to resist heat from frying (Soni et al., 2016). In addition, the spore formed by *Bacillus* sp. is reported to cause food poisons due to its ability to produce spore that damage its host and hence initiate non gastrointestinal infections (Leong et al., 2023).

Conclusion

The study has shown that microbial profiling of the fried foods is associated with *Bacillus* spp. and *Proteus* sp., all of which are aerobic bacteria, thus, the challenge thrown-off can simply not be over emphasized as *Bacillus* sp. spore formation in fried food threatens the increasing economic value of the fried foods sold in evening markets along road-side table-stands.

Recommendation

The study recommends effective preparatory measures to be adopted in cooking or frying foods for public consumption. Hence, street table vendors of fried foods should operate under proper sanitary and hygienic environmental condition.

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