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Bacteriological analysis of OKPA (Bambara nut Moi Moi) sold in NKWO market of Okija town near Legacy University Okija.

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Abstract

This research was conducted to ascertain the bacterial content of Okpa (Bambara nut moi moi) sold in Nkwo market in Okija town. This market is strategic as it is a popular market serving Legacy and Madonna Universities and the entire Okija town. Five samples were collected and analyzed using spread plate technique after tenfold serial dilution. Nutrient agar was used to enumerate total colony count while MacCokey agar was used for coliform count. After incubation for 24 hours at 37 °C, 1.2×10^5 , 2.1×10^4 , 4.5×10^4 and 2.0×10^4 cfu/g were observed for nutrient agar. For the coliform count, 1.1×10^2 , 2.1×10 , 1.2×10 and 1.0×10^2 cf/g were observed on MacConkey agar. The results showed that, though the Okpa were contaminated, the coliform count was not at the level of causing disease. Also, the research revealed that the sources of the pathogenic bacteria are through unhygienic actions of the people that prepares the food and those that hawk them. The research also recommended that adequate sanitary practices and procedures should be adopted during Okpa preparation.

Keywords: Okpa; Coliform; Dilution; Spread-plate; Incubation

1 Introduction

Okpa is an Igbo local Moi Moi mainly eaten when it is hot. It is mainly attributed to be a native of Enugu state in Igbo land. It is produced and processed from Bambara Nut (*Mkpuru Okpa*). Bambara groundnut (*Vigna subterranean*) is an indigenous legume of African origin, mainly cultivated by subsistence farmers. The seed is very nutritious but underutilized legume, which is potentially very rich in proteins and minerals (Okpuzor, Ogbunugafor, Okafor, & Sofidiya, 2010). Bambara groundnut (*Vigna subterranean*) is a tropical pulse (with underground pods), and is one of the legumes of Papilionaceae sub family. It is a small herb with trifoliolate leaves, which are palatable to domestic animals. The crop is indigenous to tropical Africa (Olapade and Adetuyi, 2007). It originated in the Sahelian region of present day West Africa, from the Bambara tribe near Timbuktu, who now live mainly in central Mali (Nwana et al., 2005), hence its name Bambara groundnut. The nuts are also known as Jugo beans (South Africa), Ntoyo Cibemba (Republic of Zambia), Gurjiya or Kwaruru (Hausa, Nigeria) Okpa (Igbo Nigeria), Epa-wro (Yoruba, Nigeria) and Nyimo beans (Zimbabwe). In many regions of Africa, Bambara ground nut is the third most important legume after groundnut and cowpea. Of the total annual production of around 330,000 tonnes, approximately half is produced in West Africa (Bamishaiye et al., 2011). There is a popular saying that you have not tasted Okpa until you eat Okpa from Enugu (Okpa Nsukka). Depending on what is available at hand, you can wrap Okpa using fresh smoked banana or plantain leaf, nylon bag,

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plastic plates/bowl etc, The leaves are preferable because it gives a certain taste to the Okpa, others might decline because the colour of the leaves penetrates into the Okpa, which reduces the yellowish colour.

2 Materials and methods

2.1 Sample collection, setting and analysis

Nkwo market is located at about 5°54'33N and 6°50'27E of Ihiala LGA in Anambra state. It is the closest market to Legacy and Madonna Universities in Okija Town. Students from Legacy University visit the market to buy food. The samples for this research were collected directly from the Okpa hawkers. Five different wraps were bought from the women without telling them what the sample will be used for. The women were asked to slice the Okpa and put it into a custard container sterilized by washing with soap, rinsed with clean water and later rinsed again with 95% ethanol. The samples were transported to Microbiology laboratory of Legacy University Okija within 45 minutes for analysis. The samples were labelled as KP1, KP2, KP3, KP4 and KP5.

2.2 Media preparation

Sterilization of Conical Flask, test tubes, Petri dishes and all the glass wares needed for the experiment was carried out in the laboratory. The glasswares were sterilized in the oven at 120°C for an hour after washing with Soap; while other equipment was surface sterilized with 70% ethanol to reduce microbial contamination (Agris, 2005). The Media Nutrient Agar, MacConkey Agar, were Prepared following the manufacturers guide. The media were allowed to cool to about 45 – 50 °C before dispensing into pre-sterilized petri dishes which were then placed on flat surface to solidify (Cheesbrough, 2010),

2.2.1 Nutrient Agar

Twenty-eight (28) grams of powdered commercially prepared of nutrient agar was weighed on electronic balance into a clean dry 1-liter conical flask and 1000 ml of distilled water placed inside a water bath for about 90°C, all the agar to dissolve and autoclave at temperature of 121 °C for 15 minutes.

2.2.2 MacConkey Agar

Forty-eight (48) grams of MacConkey Afar was weighed into 1litre capacity of colonized flask and bring to boil using water bath at temperature of about 90 °C to dissolve the agar and autoclave.

2.3 Sample preparation

Exactly one gram of Okpa was homogenized in nine (9) ml of sterile distilled water. Tenfold serial dilution was done according to Cappucino and Sherman (2016). And the 10³, 10⁴, 10⁵, 10⁶ and 10⁷ diluents were inoculated into the nutrient and MacConkey agar. Spread plate method of isolation was used. The inoculations were done in duplicates. These plates were then incubated at 37 °C for 24 hours.

3 Results

In this research, a total of five samples of Okpa were analyzed and out of the five samples, four (4) different bacterial isolates were isolated and were identified as *Staphylococcus aureus*, *Escherichia coli*, *Enterococci* and *Bacillus sp.* The table 1 below shows the biochemical characteristic, morphological features and the probable organism. Also, the Table 2 Shows the mean value for total colony count of bacterial isolates. These four bacterial isolates; *Enterococci* *Escherichia coli*, *Staphylococcus aureus* and *bacillus cereus* were mainly associated with food poisoning because of its ability to produce toxins.

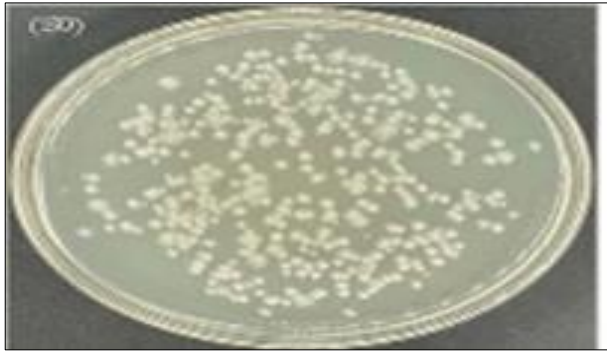


Figure 1 Growth on Nutrient Agar Plate



Figure 2 Growth MacConkey plate

Table 1 Colony Counts in respective dilution factor

Samples	Total colony count(TCC)	Coliform count (CC)
KP1	120000	110
KP2	21000	21
KP3	45000	12
KP4	2000	10
KP5	130	11

Table 2 Mean of Total and Coliform count in cfu/g per samples collected

Samples	Mean Total Colony count (Cfu/g) (Nutrient agar)	Mean Coliform count (cfu/g) MacConkey Agar
KP1	1.2×10^5	1.1×10^2
KP2	2.1×10^4	2.1×10^1
KP3	4.5×10^4	1.2×10^1
KP4	2.0×10^4	1.0×10^2
KP5	1.3×10^2	1.1×10^1

Table 2 Morphology and Biochemical analysis of Isolates

Isolates	Grams Rxn	Shape	Catalase	Oxidase	Morphology	Possible organism
1	+ve	Cocci	-ve	-ve	Small, red-purple	<i>Enterococcus faecalis</i>
2	+ve	Cocci	+ve	-ve	Golden yellow, circular	<i>Staphylococcus aureus</i>
3	-ve	Rod	-ve	+ve	Pink, circular	<i>E.coli</i>
4	+ve	Rod	+ve	-ve	Creamy, rough edge	<i>Bacillus sp</i>

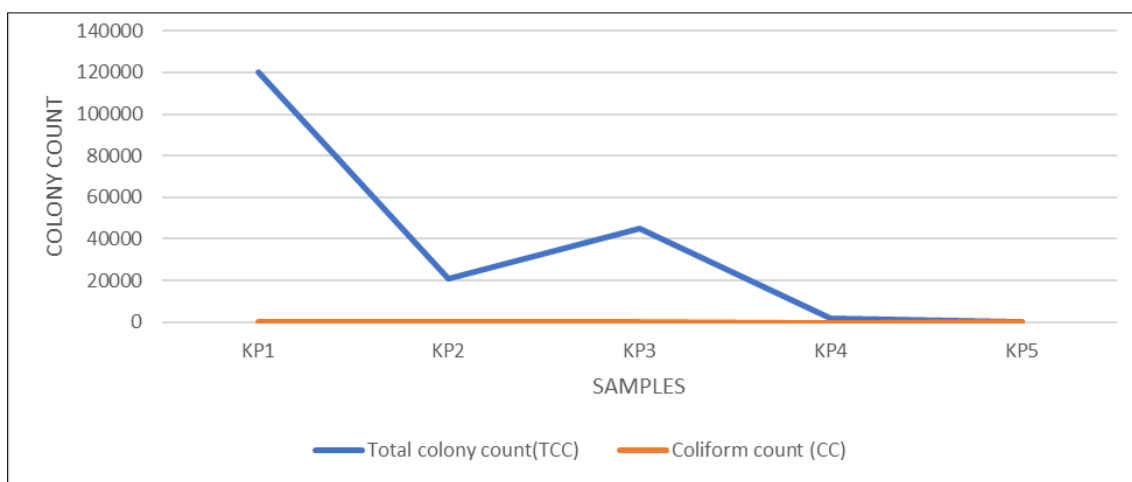


Figure 3 Graph of Total Colony and Coliform count per sample

4 Discussion

This research revealed the presence of four (4) bacteria, *Enterococci*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus sp.* The coliform level from the graph is almost at zero level. This explains why people don't fall sick even after eating the contaminated Okpa. The other total colony count which is very high are not pathogenic. This has been reported by many authors. The presence of microbial contaminants (bacteria) has been reported in ready-to-eat food in the previous works of Agao & Atere (2009) in which the bacteriological assessment and hygienic standards of food canteens in Kwara State Polytechnic, Ilorin, Nigeria was carried out. Though Okpa is a cooked food which ordinarily should be bacteria-free, but the unhygienic actions of the hawkers contribute in no small measure to the contamination of the food product. The palm oil in Okpa is believed to have antibacterial ability but human activities during selling and buying makes it impossible. These organisms are known as foodborne pathogens and opportunistic pathogens that have been implicated in foodborne disease outbreaks (Mudgil *et al.*, 2004; Oranusi *et al.*, 2007, Oranusi and Braid 2012; Tambeker *et al.*, 2008). Contaminated ready-to-eat foods are regarded as potentially hazardous to public health. The following unhygienic actions of the hawkers are contributory to the contamination of Okpa.

- Use of unwashed knife to slice the Okpa when packaging it for the buyer.
- Use of dirty cloth to wipe the knife.
- Most hawkers talk as they slice the Okpa for the buyer just to convince one that the food is good.
- The dirty container used in conveying it around.
- As they hawk it around, there is the possibility of environmental contamination
- After preparation using local plantain leaf, some hawkers again wrap the Okpa with Nylon bag which cannot be bacteria-free.
- During hawking, some buyers will lift the Okpa with their hands which may be contaminated. In the process of not been satisfied, they drop it and go their way. These buyers oftentimes are in a moving vehicle.

In some cases, Foods and ingredients are exposed to contamination from unwashed hands and material used for wrapping, such as leaves and polythene bag (Agwa *et al.*, 2012). That these bacteria are found in Okpa shows that there are unhygienic, nonsanitary practices during the production, processing and packaging of the Okpa food products and poor properly procedures in cooking.

5 Conclusion

Some food pathogens are of soil or intestinal origin. The way through which they find their way to prepared Okpa, is surprising. Also, they are transmitted through poor food preparation, personal hygiene or public sanitation practices. These enumerated points stand as a great threat to the human health as these organisms can cause disease and even death if not handled well. Therefore, to ensure the safety of the foods like Okpa, the following should be considered

- Proper handling during the preparation process should be in place.
- Preparation should be in clean environment,
- The Okpa preparation should be done using clean water.

- When the need of using, nylon becomes inevitable, clean nylon should be used.
- The plantain leaves should be smoked before use to kill surface bacteria.
- Use of clean Utensils and adoption of proper hygiene will also reduce the incidence of occurrence of these organisms.

Recommendation

Since Okpa is enjoyed when it is hot, this research is recommending that Okpa can be refrigerated as a way of preserving it. It should then be warmed with microwave when one wants to eat it.

Future prospect

Since Okpa is hawked hot and still bacteria contaminations are being reported. Research should be carried out to find out how to get rid of these pathogens.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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