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Isolation and Identification of Bacteria Associated with Beans Cake (Kosai) Sold in Adamawa State University Mubi

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ABSTRACT

This study investigated the microbial contamination of bean cake samples collected from different locations in Adamawa State University, Mubi. A total of 18 samples six samples from each location, were collected and analyzed for microbial contamination using three methods: hand picking into a clean container, hand picking into a clean container with hand wrapped in clean nylon, and picking with a fork into a clean container. The results showed a high level of microbial contamination in the bean cake samples, with Staphylococcus spp. being the most frequently isolated microorganism, followed by Escherichia coli, Streptococcus spp., Salmonella spp., Bacillus aureus, and Bacillus subtilis. The study also characterized and identified six bacterial isolates based on physical and biochemical properties. The frequency of occurrence of microbial isolates from bean cake samples was determined, and the results showed a high microbial load in the samples, ranging from 300 x 10^6 to 730 x 10^6 CFU/g. The study reveals that bean cake samples have a high frequency of microbial occurrence, indicating a risk of foodborne ses. The use of a fork for picking samples seemed to reduce the level of contamination in some cases. The findings of this study are consistent with those of other authors, who have reported high levels of microbial contamination in bean cake samples and the presence of pathogenic microorganisms. This study highlights the need for proper handling and processing of food to prevent contamination and ensure public health safety.

Key Words: Bacteria, Beans-Cake (Kosai) Adamawa, State, University, Mubi

INTRODUCTION

Cowpea (*Vigna unguiculata*) is a legume popularly known as beans in West Africa (Moutaleb *et al.,* 2017). Cowpea also contains reasonably high amount of carbonhydrates. Cowpea has about 25% protein, making it extremely valuable for people who cannot afford proteins from animal sources such as meat and fish (Appiah *et al.,* 2011; Oumarou *et al.,,* 2017). Cowpea can be processed as flour, paste, deep fried cake (‘akara’) or steam bean pudding (‘moi moi’) and bean soup eaten in several Western and Central African countries (Eke-Ejiofor & Kporna, 2019).

Bean cake known as “àkàrà” in Yoruba, “kosai” in Hausa, is a popular food in Nigeria, Ghana, Togo, Benin, Mali and Gambia (Aviara *et al.,* 2018). It forms part of the diet of most ethnic groups in Nigeria. Nigerians usually eat it as breakfast with ‘ogi’, or lunch with ‘gari’ or even dinner with ‘eko’. Akara is a traditional African food made by deep frying cowpea paste that has been whipped and seasoned with salt, pepper, onions and other optional ingredients. The outer crust of okara is crispy and the interior is spongy like bread. It is considered to be the most commonly consumed cowpea-based food in West Africa. Akara is made mainly from cowpea and other sources like maize and rice flour. It can be fried with vegetable oil, palm oil, and other edible oils (Aviara *et al.,,* 2018).

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Foodborne pathogens are microorganisms found in foods that are capable of causing diseases when consumed. Some foodborne microbes make people ill by forming toxins in foods that affect the gut or the neurological system. Foodborne pathogens include *Salmonella typhimurium, Escherichia coli, Listeria monocytogenes, Clostridium perfringens, Bacillus subtilis, Bacillus aureus* and *Klebsiella pneumoniae*. Foodborne pathogens associated with bean cake according to the findings of Lateef *et al.,* (2016) are *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Citrobacter freundii, Serratia marcescens, Proteus vulgaris, Bacillus aureus, Streptococcus pyogenes, Aspergillus niger, Shigella* spp and *Bacillus* spp. The contamination of bean's cake is largely due to post-processing operations such as unhygienic handling of bean cake with bare hands, exposing bean cake without covering it, talking when selling bean cake, wrapping/picking the bean cake in old newspapers that were not properly kept among others. These post-processing operations can be abated through the use of quality water, high level of personal hygiene and hygienic production materials (Toledo, 2018).

Examples of foodborne diseases caused by these organisms include; Salmonellosis and foodborne illness caused by *E. coli*. Other foodborne diseases include: Amoebiasis, cholera, diarrheagenic *E.coli*, giardiasis, listeriosis, marine toxins shigellosis, travelers' diarrhea, trichinosis (*trichinellosis*), typhoid fever, *Vibrio parahaemolyticus* and *Vibrio vulnificus* infection. Symptoms of these diseases include nausea, vomiting, abdominal cramps and diarrhea (Toledo, 2018).

One of the major problems associated with bean cake is its susceptibility to various types of spoilage such as staling, rancidity and ropiness, soon after its production. Bean cake starts to stale the minutes it leaves the fryer which makes its outer surface to become firm, harsh, opaque and crumblier. Bean cake has a poor shelf-life which has been attributed to its fat and high moisture content (Oumarou *et al.,* 2017). Associated with the fat content is lipid oxidation while high moisture content in the product predisposes carbohydrate and protein in it to fermentation and putrefaction respectively causing ropiness by *Bacillus subtilis* (Oumarou *et al.,* 2017).

The presence of microorganisms in food has always been attributed to contamination through water, soil, processing equipment, contact surfaces and the food handlers (Toledo, 2018). Improper handling of food is responsible for most cases of food borne diseases and cross contamination (Toledo, 2018). About 1 in 10 people in the world fall sick after eating food contaminated through farming with chemicals, processing and preservation. In Nigeria, more than 200,000 persons die of food poisoning annually caused by the consumption of contaminated foods (Aviara *et al.,* 2018).

The nutritional content of bean cake has predisposed it to microbial contamination.

There are multiple sources of contamination from the environment and contaminants could enter the food during harvest, production, storage and selling of bean cake. And as such, it is imperative that bean cake, a cowpea base delicacy be examined in Adamawa State University, Mubi for the presence of contaminants. The study aimed at isolation and identifying the microorganisms associated with bean cake sold in Adamawa State University, Mubi.

Several studies have reported the contamination of bean cake by pathogenic organisms from diverse sources Lateef *et al.,* (2016). But no report in Adamawa State University, Mubi that investigated the microbiological examination and identification of hazards and critical control points of akara. Also, Ajibola and Adelekan (2017) investigated the storage stability of deep-fried cowpea products (akara) incorporated with soy-flour and *Aframomum danielli*, while China *et al.,* (2019). Studied on the proximate composition and sensory assessment of beans pudding prepared using two different cooking methods hence, there is a great need to determine the microbial quality and identification of hazards and critical control points of bean cake sold in Adamawa State University, Mubi.

MATERIALS AND METHODS

Study Area

This study was carried out in Adamawa State University, Mubi. Mubi lies at latitude of 10°15'37"N and longitude of 13°15'38"E. The estimated population of Mubi North Local Government Area is put at 188,301 inhabitants with the area mostly populated by members of the Gude, Nzanyi and Fali ethnic groups. Mubi North Local

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Government Area has a number of notable landmarks such as the Adamawa State University and the Federal Polytechnic Mubi. (Adebayo and Tukur, 2004).

Sample Collection

A total of 6 bean cake balls were collected in each sales point using three methods; hand picking of bean cake into a clean container, hand picking (with hands wrapped in nylon) into a clean container and picking with fork into a clean container. A total of 18 balls of bean cake were collected from 3 sale points. The samples were collected and taken immediately for analysis in the Zoology Department Laboratory, Adamawa State University, Mubi. The samples were collected from three sales points in Adamawa State University, Mubi by adopting the method outlined by (Aibinu *et al.,* (2017).

Determination of Microbial Contamination Level

Pour plate method of Sagar, (2019) was used for the isolation, enumeration and identification of bacterial and fungal species contaminating the samples. A gram of each of the bean cake samples (mashed) was transferred into 9 mL of sterile distilled water to obtain a stock solution. A milliliter (1 mL) of the stock solution was transferred into 9 ml of sterile distilled water to obtain 10-1 dilution. Further dilution was made to 10-6. An aliquot (1 mL) of the 4^{th} diluent was aseptically inoculated in triplicates into freshly prepared molten nutrient agar, MacConkey agar, Salmonella Shigella agar and Manitol salt agar for bacterial count and the 3rd diluent was aseptically inoculated in triplicate into Sabouraud dextrose agar respectively. The inoculated plates were incubated for 24 hours at 37 ℃ for bacteria and at room temperature (25±2 ℃) for 48 h for fungi. The isolates were further sub-cultured repeatedly onto a fresh Nutrient agar plates for the bacteria and saboraud dextrose agar for the fungi so as to obtain pure isolates. After sub-culturing, the plates were incubated accordingly. The pure isolates was preserved in slant bottles for further characterization and identification as outlined in Bergey's Manual of Determinative Bacteriology.

The resulting colonies was counted using colony counting chamber and calculated using the formula below:

Colony forming unit (cfu) = number of colonies x volume of diluent x reciprocal of dilution

Characterization and Identification of Isolates

The method of Bassiri, (2020) was employed for the isolation and characterization of identities of the bacterial isolates. The isolates obtained was subjected to Gram staining, Motility test, Oxidase test, Catalase test, Triple sugar iron agar test, Indole test, Urease test, Hydrogen sulphide production test, Citrate utilization test, Methyl red test and Voges-prokauer test.

Gram staining

Grease-free glass slides which were used to prepare smear and the slides was placed on the staining rack. The smear was covered with crystal violet stain and left for 1 minute then was washed carefully under running tap water. The smear was flooded with Logul's iodine solution and left for 1 minute. The iodine was drained off the slides and was washed in a gentle stream of tap water. The slides was flooded with alcohol for 30 seconds and was washed under running tap water then drained completely. The slides was counterstained with safranin for 1 minute and was washed in a gentle stream of tap water until no colour appears in the effluent then the slides were blotted dry with absorbent paper and observed under the microscope. Gram-positive bacteria appeared dark purple while Gram-negative bacteria appeared pale to dark red as in (Kumar *et al.,* 2018)

Catalase test

Following the method of (Oumarou *et al.,* 2017) a sterile wire loop was used to pick colonies from 24-hour cultures on to dry glass slides. A drop of 3 % hydrogen peroxide (H_2O_2) was placed on each glass slide and observed for the evolution of air bubbles. Catalase-positive bacteria produced copious active bubbles while catalase negative bacteria produced few bubbles or none Oumarou *et al.,* 2017)

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Identification and Characterization of Fungal Isolates

Fungal isolates was identified by microscopic and macroscopic techniques as described by Onyeze *et al.,* (2013)**.** This was done by using wet mount technique to view the fungi microscopically and also checking the growth pattern, pigmentation and presence of septa for the microscopy of the fungi. The fungal isolates was identified by comparing their characteristics with those of known taxa using the schemes of Omemu *et al.,* (2018)

Determination of Frequency of Occurrence of Microbial Isolates from Bean Cake

The frequency of occurrence of the isolates was determined by counting the number of occurrences of a particular organism compared to the total organisms isolated from all the bean cake samples.

Data Analysis

Results was expressed as the mean values ± standard error of mean (SEM) by measuring three independent replicates. Analysis of variance (ANOVA) using one-way was done and Duncan's test was performed to test the significance difference between means values obtained among the treatments at 5% level of significance using SPSS software (version 21, IBM SPSS). Differences was considered significant at $p<0.05$.

RESULTS

Determination of Microbial Contamination Level

The result of this study highlights the presence of various microorganisms in beans cake samples collected from different locations. Table 1 presents the results of the study on microbial contamination levels in different beans cake samples collected from various locations.

Sample 1 (Opp. Zainab Hostel): Sample A (Hand picking into a clean container): Staphylococcus spp. and *E.coli* were identified, Sample B (Handpicking into clean container with hand inside nylon): Streptococcus spp. and Salmonella spp. were identified, Sample C (Picking with fork into clean container): Staphylococcus spp. was identified.

Sample 2 (In front of boys Hostel): Sample A (hand picking into a clean container): *Bacillus aureus* and *E. coli* were identified, Sample B (handpicking into clean container with hand inside nylon): Salmonella spp. and Staphylococcus spp. were identified, Sample C (Picking with fork into clean container): Streptococcus spp. and *Bacillus subtilis* were identified.

Sample 3 (Up commercial): Sample A (Hand picking into a clean container): *E. coli,* Streptococcus spp., and *Bacillus aureus* were identified - Sample B (handpicking into clean container with hand wrapped in nylon bag): *Bacillus aureus,* Streptococcus spp., and Salmonella spp. were identified, Sample C (Picking with fork into clean container): No bacteria were identified.

Observations: Staphylococcus spp. was identified in most samples, indicating a high level of contamination, *E. coli* was identified in Samples 1 and 3, indicating possible fecal contamination, Salmonella spp. was identified in Samples 1 and 2, indicating possible food poisoning risk, *Bacillus aureus* was identified in Samples 2 and 3, indicating possible food spoilage, Streptococcus spp. was identified in most samples, indicating possible throat and skin infections, The use of a fork for picking samples (Sample C) seemed to reduce the level of contamination in some cases.

Characterization and Identification of Isolates

The results of the characterization and identification of isolates shows six bacterial isolates based on physical and biochemical properties. The isolates are:

E. coli, *Bacillus subtillus*, Streptococcus Spp., *Staphylococcus aureus,* Salmonella spp. and *Bacillus aureus* as seen in table 2. The isolates were identified based on characteristics such as colony appearance, Gram reaction,

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oxidase test, catalase test, and mortality test. The results can be used to understand the properties and potential pathogenicity of the isolates**.**

Determination of Frequency of Occurrence of Microbial Isolates from Bean Cake in Adamawa State University, Mubi

Table 3, presents the frequency of occurrence of microbial isolates from bean cake samples, measured in colony-forming units per gram (CFU/g).

The table shows three sets of samples (1, 2, and 3), each with three replicates (A, B, and C).

The CFU/g values range from 300 x 10^6 to 730 x 10^6, indicating a high microbial load in the bean cake samples.

Sample 3A has the highest microbial load (730 x 10^6 CFU/g), followed by sample (2A, 2B and 3C) while Sample 1B has the lowest (300 x 10^6 CFU/g).

The replicates within each set show varying microbial loads, indicating potential variations in sampling or processing overall, the table suggests that bean cake samples have a high frequency of microbial occurrence.

Table 1. Determination of Microbial Contamination Level

Samples	Bacteria identified on each sample
Sample 1 (Opp. Zainab Hostel) Sample A (Hand picking into a clean container) Sample B (Handpicking into clean container with hand inside Nylon) Sample C (Picking with fork into clean container)	Staphylococus spp, *E. coli* Streptococcus spp, Salmonella spp. Staphylococcuss spp
Sample 2 (In front of boys Hostel) Sample A (Hand picking into a clean container) Sample B (Handpicking into clean container with hand inside Nylon) Sample C (Picking with fork into clean container)	*Bacillus coreus, E. coli* Salmonella spp, Staphylococcuss spp Streptococcus spp, *Subtilus*
Sample 3 (Up commercial) Sample A (Hand picking into a clean container) Sample B (Handpicking into clean container with hand inside Nylon) Sample C (Picking with fork into clean container)	*E. coli*, Streptococcus spp, *Bacillus aureus* *Bacillus aureus* , Streptococcus spp, Salmonella spp.

Table 2. Characterization and Identification of Isolates

Sample	Characteristics of colonies	Gram staining reaction	Oxidase test	Catalase test	Mortality test	Name of organism
1	Appears whitish grey with smooth moist surface and clear margin. Display white to greyish	Gram positive Cocci appear single and in short chain	-	+	motile	*E. coli*

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	colors surrounded by wide zone of beta hemolysis on blood agar.					
2	Yellow round colony on MHA appear white creamy surrounded by clear zone opaque of beta hemolysis on blood agar	Gram positive cooci appear in cluster and single	-	+	motile	*Baccillus subtilus*
3	Dome shaped greyish white colony with smooth surface display pinkish to white color surrounded by clear zone of beta haemolysis on blood agar.		+	+	Non motile	Streptococcus Spp.
4	Yellow round large corvex colony on MHA appear white creamy surrounded by clear opaque zone of beta haemolysis on blood agar	Gram positive cocci in chesters and single	-	+	Non motile	*Staphylococcus aureus*
5	Greyish white colony that is not hemolytic on blood agar medium appear white and slightly moist appearances with tailing end in nutrient agar media	Gram positive cocci in chain pairs and single	-	+	Non motile	Salmonella spp
6	Appears pinkish on NA, greyish white with clear blood zone of beta haemolysis on blood agar	Gram positive baccili in pairs and shot chain	-	+	motile	*Bacillus aureus*

Table 3. Determination of Frequency of Occurrence of Microbial Isolates from Bean Cake

Sample	Colony forming unit per gram
1A	**370 X 10^6 Cfu/g**
1B	**300 X 10^6 Cfu/g**
1C	**530 X 10^6 Cfu/g**
2A	**700 X 10^6 Cfu/g**
2B	**700 X 10^6 Cfu/g**
2C	**620 X 10^6 Cfu/g**
3A	**730 X 10^6 Cfu/g**
3B	**460 X 10^6 Cfu/g**
3C	**700 X 10^6 Cfu/g**

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DISCUSSION

The results of this study highlight the presence of various microorganisms in bean cake samples collected from different locations in Adamawa State University, Mubi. The researchers used three methods of sampling: hand picking into a clean container, hand picking into a clean container with hand inside nylon, and picking with a fork into a clean container. The results show that Staphylococcus spp. was the most frequently isolated microorganism, followed by *E. coli* and Streptococcus spp. This agree with the findings of Omemu *et al.,* (2018), who reported a high prevalence of Staphylococcus spp. in bean cake sold in markets in Lagos, Nigeria. Similarly, a study by Ayodele *et al.,* (2019) found that *Staphylococcus aureus* was the most common isolate from bean cake samples in Ibadan, Nigeria. Microorganisms such as *E. coli*, Salmonella spp. and *Staphylococcus aureus* were also found in the bean cake.

The study also characterized and identified six bacterial isolates based on physical and biochemical properties. The isolates were identified as *E. coli, Bacillus subtilis,* Streptococcus spp., *Staphylococcus aureus,* Salmonella spp. and *Bacillus aureus*. The presence of pathogenic samples is a concern for public health, as these microorganisms can cause foodborne diseases (World Health Organization, 2018). This is in agreement with the findings of other studies, which have reported the presence of pathogenic microorganisms in bean cake samples (Aibinu *et al.,* 2017; Omemu *et al.,* 2018).

The frequency of occurrence of microbial isolates from bean cake samples was determined, and the results showed a high microbial load in the samples, ranging from 300 x 10^6 to 730 x 10^6 CFU/g (Table 4.3). This is in line with the findings of Omemu *et al.,* (2018), who reported high levels of microbial contamination in bean cake sold in markets in Lagos, Nigeria. However, the microbial load found in this study is higher than that of Ayodele *et al.,* (2019), who found a microbial load ranging from 100 x 10^6 to 500 x 10^6 CFU/g in bean cake samples in Ibadan, Nigeria.

The result of this study reveals that there are high levels of microbial contamination in beans cake samples in Adamawa State University, Mubi. The findings of this study are concur with the results of (Aibinu *et al.,* 2017; Omemu *et al.,* 2018; Ayodele *et al.,* 2019), who reported high levels of microbial contamination in bean cake samples and the presence of pathogenic microorganisms

In conclusion the researcher, investigated the microbial contamination of beans cake samples collected from various locations, including opposite Zainab Hostel, in front of Boys Hostel, and Up Commercial. The results showed a high level of contamination with various microorganisms, including: Staphylococcus spp., *E. coli*, Salmonella spp., *Bacillus aureus* and Streptococcus spp.

The study found that the use of a fork for picking samples seemed to reduce the level of contamination in some cases, highlighting the importance of proper food handling practices.

Based on the results of this study, the researchers proffer the following recommendations:

i. Public awareness campaigns should be organized to educate food processors, handlers, vendors and consumers on food safety and hygiene practices.

ii. Further research should be conducted to identify specific microorganisms present in other food samples and their potential risks.

REFERENCES

1. Ajibola, F. C. & Adelekan, F. (2017). Storage Stability of Deep-fried Cowpea Products (akara) Incorporated with Soy-flour and Aframomum danielli. British Journal of Applied Science & Technology, **8**(2): 204-212.
2. Alum, E. A., Urom, S. M., Chukwu, B. & Chukwu M. A. (2016). Microbiological Contamination of Food: The Mechanisms, Impacts and Prevention. International Journal of Scientific and Technology Research, **5**(03): 3-7.

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3. Appiah, F., Asibuo, J. & Kumah, P. (2011). Physicochemical and functional properties of bean flours of three cowpea (Vigna unguiculata L. Walp) varieties in Ghana. African Journal of Food Science, **5**(2): 100–104.
4. Aviara, N. A., Olarinde, M. O., Nasirudeen, A. R., Lasisi, L. A., Balogun, S. O., & Ogunsola, F. O. (2018). Empirical analysis of traditional akara frying process. Arid zone Journal of Engineering, Technology & Environment, **14**(4): 225-236.
5. Bassiri, E. A (2020). Antimicrobial susceptibility Testing. Retrieved from https://www.sas.upenn.edu. 18th July 2021.
6. China, M., Nua, J., Deedam, P. M. & Christabel, P. (2019). Proximate composition and sensory assessment of beans pudding prepared using two different cooking methods. Research Journal of Food Science and Nutrition, **4**(2): 58-64.
7. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Journal of Molecular Biology and Evolution, 35, 1547-1549.
8. Lateef, T. E., Davies, A., Adelekan, I. A., Adelere, A. A. & Fadahunsi, A. H. (2016). Akara Ogbomoso: Microbiological Examination and Identification of Hazards and Critical Control Points. International Journal of Food Science and Technology, 16,386-389.
9. Moutaleb, O. H., Amadou, I. & Amza, T. (2017). Physico-functional and sensory properties of cowpea flour-based recipes (akara) enriched with sweet potato. Journal on Nutritional Health Food Engineering,**7**(4): 325–330.
10. Oumarou, H. M., Issoufou, A. & Tidjani A., (2017). Physico-functional and sensory properties of cowpea flour based recipes (akara) and enriched with sweet potato. Journal of Nutritional Health & Food Engineering, **7**(4): 85 -91.
11. Sagar, A. (2019). Pour plate Technique: Procedure, Advantages and Limitations. Retrieved from https://microbenotes.com
12. Toledo, R. T. (2018). Fundamentals of Food Process Engineering, Springer International Publishing AG, part of Springer Nature. Retrieved from https://doi.org/10.1007/978-3-319-90098-8-9.