**ISOLATION AND CHARACTERIZATION OF MICROORGANISMS ASSOCIATED WITH BEAN CAKE SOLD IN MINNA METROPOLIS**

Bean cakes are ready-to-eat deep-fried cowpea paste. It is commonly known as àkàrà or kosai in Nigeria. Analysis of fried bean cakes was carried out to isolate and characterize the microorganisms associated with bean cakes sold in Minna Metropolis. The Frequency of occurrence of the isolates were also determined. The isolates identified by 16S rRNA molecular technique were *Aspergilus niger, Bacillus subtilis, Micrococcus roseus, Staphylococcus aureus, Klebsiella pneumonia* and *Escherichia coli.* The total viable bacterial counts ranged from 0±0.0cfu/g in Dutsen kura to 2.58x105±1.00x104cfu/g in F-layout while the coliform counts ranged from 0±0.0 cfu/g in Bosso and Dutsen kura to 1.75x105±1.09x104 in Kpakungu and the fungal counts ranged from 0±0.0 in Tunga and Bosso to 2.66x103±1.77x102 in Maitumbi. *Staphylococcus aureus* was the most frequently isolated organisms from all the bean cakes while *Aspergillus niger* was the least. The results suggested that some degree of control can be exercised over the microbial hazard of kosai to prevent, eliminate or reduce microbial load to acceptable levels. There should be enlightenment campaign amongst sellers and consumers of bean cakes.

Keywords: Bean cakes, Microorganisms, antimicrobial discs, microbial resistance, molecular characterization.

**INTRODUCTION**

Cowpea (*Vigna unguiculata*) is a legume popularly known as beans in West Africa (Moutaleb*et al.,* 2017). Cowpea is nutritious and contains protein, vitamins and minerals. Cowpea can be processed as flour, paste, deep fried cake (akara) or steam bean pudding (moimoi) and bean soup eaten in several Western and Central African countries (Eke-Ejiofor and 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 ingredient. (Asare *et al.,* 2013).

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 (Asare*et al.,* 2013) Foodborne pathogens associated with bean cake according to the findings of Lateef*et al.* (2010) are *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Citrobacterfreundii, Serratiamarcescens, Proteus vulgaris, Bacillus cereus, Streptococcus pyogenes, Aspergillusniger, Shigella sp.* and *Bacillus sp.*

The contamination of bean 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, using 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 (Lateef *et al.,* 2010).

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 (Ikya *et al.,* 2013). 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* (Ikya *et al.,* 2013). In view of the problems associated with the consumption of bean cake, particularly if basic hygienic considerations are not observed after its production, this study was designed to evaluate the microbiological quality of bean cake from some selected points within Minna metropolis.

**Materials and Methods**

**Sample Collection**

A total of 270 bean cake balls were collected in this study using three methods; hand picking of bean cake into nylon, hand picking into clean container and picking with fork into clean container. A total of 252 balls of bean cake were collected from 14 sale points and 18 bean cake balls were prepared aseptically in the laboratory. The samples were collected and taken to the laboratory immediately for analysis. In addition, grinded bean paste and water samples were collected from these points for analyses. The samples were collected from two sales points in Tunga, Maitumbi, Mobil, F-layout, Dutsen kura, Kpagungu and Bosso areas of Minna, Nigeria. The samples were collected in the months of January, February and March, 2020. Figure I shows the picture of bean cake.

Figure I: Picture of bean cake (Field Trip)

**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) were transferred into 10 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 4th 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 innoculated plates were incubated for 24 - 48 hours at 37 for bacteria and at room temperature (252for 48-72 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 were preserved in slant bottles for further characterization and identification as outlined in Bergey’s manual of determinative bacteriology.

The resulting colonies were counted using coulter 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 Bala *et al.* (2011) was employed for the isolation and Characterization of identities of the bacterial isolates. The isolates obtained were subjected to Gram staining, Motility, Oxidase, Catalase, Triple sugar iron agar, Indole, Urease, Hydrogen sulphide production, Citrate utilization, Methyl red and Voges-prokauer tests. The fungal isolates were 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 macroscopy of the fungi. The fungal isolates were identified by comparing their characteristics with those of known taxa using the schemes of Salako (1994). All the isolates were subjected to molecular characterization.

**Molecular Identification of Isolates**

DNA was extracted using the protocol stated by Tamura and Nei (1993). Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28 ºC. After this period, cultures were centrifuged at 4600 revolution per minute (rpm) for 5 minutes. The resulting pellets were re-suspended in 520-μl of TE buffer (10 mMTrisHCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20% SDS and 3μl of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hour at 37 ºC, then 100μl of 5 M NaCl and 80-μL of a 10% CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 min at 65 ºC and kept on ice for 15 minutes. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 minutes and centrifugated at 7200rpm for 20 minutes. The aqueous phase was then transferred into a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20 ºC for 16 h. The DNA was collected by centrifugation at 13000rpm for 10 minutes, washed with 500-μl of 70% ethanol, air dried at room temperature for approximately three hours and finally dissolved in 50-μl of TE buffer.

The PCR sequencing preparation cocktail consisted of 10µl of 5x GoTaqcolourless reaction, 3µl of 25mM MgCl2, 1-µl of 10 mM of dNTPs mix, 1µl of 10 pmol each 27F 5’ AGA GTT TGA TCM TGG CTC AG3’ and 1525R, 5′AAGGAGGTGATCCAGCC3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42-µl with sterile distilled water 8μl DNA template. The PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by 30 cycles consisting of 94°C for 30 s, 50°C for 60s and 72°C for 1 minute 30 seconds; and a final termination at 72°C for 10 mins. and chilled at 4oC.GEL (2,3).

The integrity of the amplified 1.5Mb gene fragment was checked on a 1% Agarose gel done to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 min. The molten agarose was allowed to cool to 60°C and stained with 3-µl of 0.5 g/ml ethidium bromide (which absorbed invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 min to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2-μl) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4-µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 min visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel.

The amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6-µl of Sodium acetate 3M and 240-µl of 95% ethanol were added to about 40-µl PCR amplified product in a new sterile 1.5µl tube eppendorf, mixed thoroughly by vortexing and kept at 20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol and homogenized then centrifuged for 15 min at 7500 rpm and 4°C. All supernatant was removed (by inverting tube on trash) and inverting tube on paper tissue and allowed to dry in the fume hood at room temperature for 10-15 min. Then re-suspended with 20-µl of sterile distilled water and kept at 20oC prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1h as previous, to confirm the presence of the purified and quantified product using a nano drop of model 2000 from thermo scientific.

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers’ manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio Edit software and MEGA 6 were used for all genetic analysis.

**Aseptic Preparation of Bean Cake in the Laboratory**

Bean cake was prepared aseptically. About 200 grams of Beans obtained from Kure market, Minna, was washed to remove dirt and coat. Washed pepper and onions were added to it and blended using a sterile blender (VTCL Solitaire multipurpose mixer). The blended bean paste was transferred into a clean bowl and stirred in a continuous circular pattern, salt was added (to taste). Oil of about 3 inches deep was heated and the paste was then scooped with a table spoon into the hot oil and was allowed to fry till light brown, it was then flipped to enable the top to fry too. Clean basket was used to collect the bean cake and allowed to drain, which was later transferred into a sterile container. Isolation of microorganisms from the aseptically prepared bean cake was carried out. Figure II shows the flowchart for the preparation of fried bean cake (Akara).

Fig II: Flowchart for the preparation of fried Bean cake

**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 occurrence of a particular organism compared to the total organisms isolated from all the bean cake samples.

**Statistical Analysis**

Results were 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 mean values obtained among the treatments at the 5% level of significance using SPSS software (version 21, IBM SPSS). Differences were considered significant at p<0.05.

**RESULTS AND DISCUSSION**

**Bacterial load of Bean Cakes Sold in Minna Metropolis**

Table 1 shows the effects of the different collection methods (namely, bare hand + nylon, bare hand + clean container, fork + clean container) on the bacterial counts of bean cakes sold in Minna metropolis. The bacterial counts of bean cakes sold in Tunga market were not significantly (P≥0.05) different from each other. At Bosso, the bacterial load of the bean cakes collected with fork + clean container was the least while the bacterial counts of bean cakes collected with bare hand + nylon and bare hand + clean container were not significantly (P≥0.05) different from each other.

In Dutsenkura, there was no bacterial growth in the bean cakes collected with fork + clean container while the bean cakes collected with bare hand + nylon and bare hand + clean container were not significantly (P≥0.05) different from each other but higher than that collected with fork + clean container. The bacterial counts of bean cakesagainst the mode of collection for Mobil were not significantly (P≥0.05) different from each other but they were higher than the control.

At Kpakungu, the bacterial counts of the bean cakes collected with bare hand + nylon and bare hand + clean container were not significantly (P≥0.05) different from each other but they were higher than the bacterial counts of bean cakes collected with fork + clean container.At F-layout, the bacterial counts of the bean cakes collected with bare hand + nylon and bare hand + clean container were not significantlydifferent at P ≥ 0.05 from each other, but higher than the counts for bean cakes collected with fork + clean containers. The bacterial counts of bean cakes collected from Maitumbi were significantly (P ≤ 0.05) different from each other. The bean cakes collected with fork + clean containers had the lowest bacterial counts, followed by the bean cakes collected with bare hand + clean container while the cakes collected with bare hand + nylon had the highest bacterial count.

The bacterial loadof bean cakes collected with bare hand + nylon from Tunga and Mobil were the least when compared with the other areas and the control while that of Bosso, Dutsen Kura, Kpakungu and F-layout were not significantly (P≥0.05) different from each other but higher than that of Tunga and Mobil. The microbial load of bean cakes collected with bare hand + nylon was highest in Maitumbi when compared with that of other areas and the control. The bacterial counts of bean cakes for Kpakungu were significantly (P ≤ 0.05) different from each other. The bacterial counts of the bean cakes collected with bare hand + nylon and bare hand + clean container were not significantly(P ≥ 0.05) different from each other, but higher than the bean cakes collected with fork + clean containers.

The bacterial load of bean cakes collected with bare hand + clean container inTunga and Mobil were the least when compared with other areas and the control while that of Bosso, Dutsenkura, Kpakungu F-layout and Maitumbi were not significantly (P≥0.05) different from each other but significantly (P≤0.05) higher than that of Tunga, Mobil and the control.

There was no bacterial growth in bean cakes collected with fork + clean containers from Dutsen kura when compared with other areas. The bacterial load in bean cakes collected from Dutsen kura and the control are comparable to each other. Generally, bean cakes collected with fork + clean container had the least bacterial load when compared with other mode of collection.

**Table 1: Bacterial load of Bean Cakes Sold in Minna Metropolis, Nigeria**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Mode of Collection** | **Bacterial Count (cfu/g) x105 of bean cakes from various sale points** | | | | | | | |
| **Tunga** | **Bosso** | **Dutsen Kura** | **Mobil** | **Kpakungu** | **F-Layout** | **Maitumbi** | **Control (L)** |
| Bare Hand + Nylon | 1.75±0.15a | 2.54±0.13b | 1.75±0.19b | 1.55±0.18a | 1.83±0.17b | 2.58±0.10b | 2.46±0.17c | 0.00±0.00 |
| Bare Hand + Clean Container | 1.33±0.16a | 2.16±0.15b | 1.54±0.10b | 1.16±0.14a | 2.75±0.15b | 1.75±0.19b | 1.33±0.17b | 0.00±0.00 |
| Fork + Clean Container | 1.70±0.11a | 1.66±0.11a | 0.00±0.00a | 0.42±0.14a | 0.08±0.00a | 0.33±0.12a | 0.25±0.15a | 0.00±0.00 |

Values are means ± standard error of mean of triplicate determinations. Values with the same superscript in the same column are not significantly different at p<0.05

L: Aseptically prepared bean cake

**Coliform Counts of Bean Cakes Sold in Minna Metropolis**

Table 2 shows the effects of the different collection methods (namely, bare hand + nylon, bare hand + clean container, fork + clean container) on the coliform counts of bean cakes sold in Minna metropolis. The coliform counts of bean cakes from Tunga, Mobil and F-layout were not significantly (P≥0.05) different from each other, but higher than the control. There was no coliform growth on bean cakes sold at Bosso and Dutsen kura for all the modes of collection. This result is similar to the control. The coliform counts of bean cakes sold at Mobil for all the modes of collection are not significantly (P≥0.05) different from each other but higher than the control. The coliform count of bean cake sold at Kpakungu collected with bare hands + nylon and bare hand + clean container are not significantly (P≥0.05) different from each other but higher than those collected with fork and clean container. The coliform counts of the bean cakes sold at F-layout for all the modes of collection are not significantly different from each other but higher than the control. The coliform counts of bean cakes sold at Maitumbi were significantly (P≤0.05) different from each other. The bean cake sold with bare hand + nylon had the highest coliform counts while the bean cakes sold with Fork + clean container had the least coliform counts. The coliform counts of bean cakes sold in Maitumbi were significantly (P≤0.05) higher than the control.

**Table 2: Coliform Counts of Bean Cakes Sold in Minna Metropolis, Nigeria**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Mode of Collection** | **Coliform Count (cfu/g) x105 of bean cakes from various sale points** | | | | | | | |
| **Tunga** | **Bosso** | **Dutsen Kura** | **Mobil** | **Kpakungu** | **F-Layout** | **Maitumbi** | **Control(L)** |
| Bare Hand + Nylon | 1.43±0.12a | 0.00±0.00 | 0.00±0.00 | 1.0±0.16a | 1.25±0.15b | 1.08±0.17a | 0.67±0.16c | 0.00±0.00 |
| Bare Hand + Clean Container | 0.95±0.10a | 0.00±0.00 | 0.00±0.00 | 0.42±0.12a | 1.75±0.11b | 0.83±0.01a | 0.46±0.11b | 0.00±0.00 |
| Fork + Clean Container | 0.83±0.224a | 0.00±0.00 | 0.00±0.00 | 0.33±0.11a | 0.02±0.02a | 0.25±0.18a | 0.01±0.17a | 0.00±0.00 |

Values are means ± standard error of mean of triplicate determinations. Values with the same superscript in the same column are not significantly different at p<0.05

L: Aseptically prepared bean cake

**Fungal Load of Bean Cakes Sold in Minna Metropolis**

Table 3 shows the effect of the different collection methods (namely, bare hand + nylon, bare hand + clean container, fork + clean container) on the fungal counts of bean cakes sold in Minna metropolis. There was no fungal growth on bean cakes sold in Tunga and Bosso. This result is comparable with the control. The fungal counts of bean cakes sold at Dutsen kura were significantly (P≤0.05) different from each other. The bean cakes collected with fork + clean container did not yield fungal growth while the bean cake sold with bare hand + nylon had the highest fungal count. At Mobil there was no fungal growth on the bean cakes collected with bare hands + clean container and fork + clean container. There was significant fungal growth in the bean cakes collected with bare hand + nylon. At Kpakungu, there was no fungal growth on the bean cakes collected with bare hands + clean containers as well as fork + clean container. There was significant fungal growth on the bean cakes collected with bare hands + nylon. At F-layout, the fungal count of the bean cake collected with bare hand + nylon was significantly (P≤0.05) different from the bean cakes collected with bare hands + clean container and fork + clean container. There was no fungal growth from the bean cakes sold with bare hands + clean container and fork + clean container.

The fungal counts on bean cakes sold with bare hand + nylon at Dutsen kura, Mobil, Kpakungu, F-layout, and Maitumbi are not significantly different from each other but higher than the control. There was no fungal growth on the bean cakes collected from Tunga and Bosso which are the same with that of the control. There was no fungal growth on the bean cake collected with bare hand + clean container at Tunga, Bosso, Mobil, Kpakungu, F-layout and Maitumbi which is the same with that of the control. The fungal counts of the bean cakes collected with bare hand + clean container at Dutsen kura was significantly (P≤0.05) higher than the other areas. There was no fungal growth in the bean cakes collected with fork + clean container from all the areas which is similar to that of the control.

**Table 3: Fungal Load of Bean Cakes Sold in Minna Metropolis, Nigeria**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Mode of Collection** | **Fungal Count (cfu/g) x103 of bean cakes from various sale points** | | | | | | | |
| **Tunga** | **Bosso** | **Dutsen Kura** | **Mobil** | **Kpakungu** | **F-Layout** | **Maitumbi** | **Control(L)** |
| Bare Hand + Nylon | 000±0.00 | 0.00±0.00 | 2.00±0.11b | 8.33±0.17b | 2.00±0.11b | 2.33±0.19b | 2.66±0.18b | 0.00±0.00a |
| Bare Hand + Clean Container | 0.00±0.00 | 0.00±0.00 | 0.83±0.11c | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a |
| Fork +Clean Container | 0.00±0.00 | 0.00±0.00 | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a |

Values are means ± standard error of mean of triplicate determinations. Values with the same superscript in the same column are not significantly different at p<0.05

L: Aseptically prepared bean cake

**Morphological Characteristics of Bacterial Isolates**

The morphological characteristics of the bacterial isolates obtained on both nutrient and MacConkey agar are shown in Table 4. The organisms isolated include *Bacillus subtilis, Micrococcus roseus, Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli.*

**Biochemical Characteristics of Bacteria Isolated from Bean Cakes**

The microscopic and biochemical characteristics of the bacterial isolates are shown in Table 5.*Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus*, and *Escherichia coli* were oxidase negative while *Micrococcus roseus* was oxidase positive. All the test isolates were catalase positive. *Bacillus subtilis, S. aureus* and *M. roseus* were Gram-positive while *K. pneumoniae* and *E. coli* were Gram-negative. *Bacillus subtilis, K. pneumoniae, E, coli* and *M. roseus* were coagulase negative while *S. aureus* was coagulase positive. *Bacillus subtilis, K. pneumoniae, S. aureus* and *M. roseus* were indole negative while *E. coli* was indole positive. *Bacillus subtilis, K. pneumonia* and *M. roseus* were methyl red negative while *S. aureus* and *E. coli* were methyl red positive. *Bacillus subtilis, K. pneumoniae* and *S. aureus* were positive to both Voges-Proskauer and citrate utilization test while *E. coli* and *M. roseus* were negative to the test. *Bacillus subtilis,* and *E. coli* were negative to urease test while *K. pneumoniae, S. aureus* and *M. roseus* were urease positive. *Bacillus subtilis, K. pneumonia*e, *S. aureus and E. coli* did not produce hydrogen sulphide gas while *M. roseus* produced the gas.

**Morphological Characteristics of Fungal Isolates from bean cake**

One genera of mould namely, *Aspergillus* was isolated from the bean cake on the basis of its colonial morphology, and microscopic characteristics. The identity of the fungus was cross matched with known taxa. Table 6 shows the morphological characteristics of fungal isolatefrom bean cake. Black colonies were isolated on saboraud dextrose agar. And on microscopic observation, rough walled septate hyphae, dark brown to black colour were seen.

**Frequency of Occurrence of the microorganisms isolated from bean cake sold in Minna Metropolis**

*Staphylococcus aureus* was the most frequently isolated organisms from all the bean cakes while *Aspergillus niger* was the least. Table 7 shows the frequency of occurrence of the microorganisms isolated from bean cake sold in Minna Metropolis.

**Table 4: Cultural Characteristics of the Bacterial Isolates**

|  |  |  |
| --- | --- | --- |
| Nutrient Agar | MacConkey Agar | Suspected organisms |
| Large, slimy colony no pigmentation and very mucoid | Large, slimy colony; pink center and very mucoid | *Klebsiella pneumoniae* |
| Circular small colonies with yellow pigment | ND | *Staphylococcus aureus* |
| Large, irregular spreading colony |  | *Bacillus subtilis* |
| Circular, entire, convex with red pigmentation | ND | *Micrococcus roseus* |
| Large, thick, greyish white, smooth and translucent disc | Large, doughnut shaped and dark pink colonies | *Escherichia coli* |

ND – not determined

**Table 5: Microscopical, Morphological and Biochemical Characteristics of the Bacterial Isolates**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| GR | SHAPE | COG | OX | CT | IN | MR | VP | CI | UR | HSS | Suspected organisms |
| + | Rod | \_ | \_ | + | \_ | \_ | + | + | \_ | \_ | *Bacillus subtilis* |
| \_ | Rod | \_ | \_ | + | \_ | \_ | + | + | + | \_ | *Klebsiella pneumoniae* |
| + | Cocci | + | \_ | + | \_ | + | + | + | + | \_ | *Staphylococcus aureus* |
| \_ | Rod | \_ | \_ | + | + | + | \_ | \_ | \_ | + | *Escherichia coli* |
| + | Cocci | \_ | + | + | \_ | \_ | \_ | \_ | + | - | *Micrococcus roseus* |

GR: Gram’s reaction, COG: Coagulase test, OX: Oxidase test, CT: Catalase test, MR: Methyl red test, IN: Indole test, UR: Urease test, H2S: Hydrogen Sulphide Production test, L: Lactose sugar fermentation test, S: Sucrose sugar fermentation test, G: Glucose sugar fermentation test, VP: VogesProskauer test, CI: Citrate utilization test, +: Positive, -: Negative

**Table 6: Cultural and Morphological Characteristics of Fungal Isolates from Bean Cake**

|  |  |  |
| --- | --- | --- |
| Cultural characteristics | Microscopic characteristics | Organisms suspected |
| Black colony on SDA | Septate hyphae, dark brown to black and rough walled | *Aspergillus niger* |

**Table 7: Frequency of Occurrence of Bacterial isolates of Bean Cakes sold in Minna Metropolis**

|  |  |  |
| --- | --- | --- |
| Microorganisms | Number | % Frequency of occurrence |
| *Staphylococcus aureus* | 60 | 23.08 |
| *Escherichia coli* | 53 | 20.38 |
| *Bacillus subtilis* | 45 | 17.31 |
| *Micrococcus roseus* | 42 | 16.15 |
| *Bacillus megatarium* | 29 | 11.15 |
| *Klebsiella pneumoniae* | 25 | 9.62 |
| *Aspergillus niger* | 6 | 2.31 |

**4.8 Molecular Identities of the Isolates**

The molecular characterization using 16rRNA confirmed the isolates as *Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus*, *Escherichia coli, Micrococcus roseus* and *Aspergillus niger.* Table 8 shows the accession number of the isolates*.* The summary of molecular characteristics of the tests organisms are presented in figure III and IV respectively.

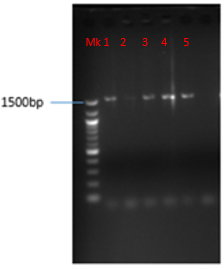


Figure III: Gel electrophlorograph showing the positive amplification of bacterial isolates using the 16S universal primers. A band size of about 1500bp indicated a positive amplification

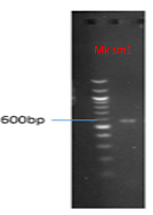


Figure IV: Gel electrophlorograph showing the positive amplification of sabr6 (fungi) isolate using the ITS universal primers. A band size of about 550bp indicated a positive amplification

**Table 8: Accession Number of the Isolates**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Scientific Name | Max Score | Total Score | Query Cover | Identity % | Accession  Number |
| *Bacillus subtilis* | 2695 | 2695 | 100% | 100.00% | MZ185316 |
| *Klebsiella pneumonia* | 2656 | 2656 | 100% | 99.79% | MZ185317 |
| *Staphylococcus aureus* | 2695 | 2695 | 100% | 100.00% | MZ185318 |
| *Escherichia coli* | 2543 | 2543 | 100% | 100.00% | MZ185319 |
| *Micrococcus roseus* | 2193 | 2193 | 99% | 99.50% | MZ185320 |
| *Aspergillus niger* | 1037 | 1037 | 98% | 100.00% | MZ171448 |

**5.0 Discussion**

In the present study, a variety of microorganisms have been isolated from bean cake based on different collection methods (namely, bare hand + nylon, bare hand + clean container, fork + clean container) Generally, bean cakes collected with Bare hands + nylon had the highest microbial load while those collected with fork + clean container had the least microbial load. This could be due to certain unhygienic practices of the producer. Examples of such unhygienic practices include: blowing air into the nylon with the mouth which may introduce oral contaminants into the nylon, using bare hands to pick bean cakes which may introduce contaminants into the bean cakes. This is similar to the findings of Lateef *et al.* (2010), where it was observed that contamination of bean cake usually occur at the post processing stage.

Coliforms were detected in the bean cakes sold in Minna metropolis. This indicates that the raw materials used for making Akara were contaminated with fecal matter. This is in agreement with the study of Sana *et al.* (2013) who observed that fecal matters might be responsible for coliform growth in a sample. The coliform counts of bean cakes from all the sale points ranged from 0±0.0 cfu/g in Bosso and Dutsen kura to 1.75x105±1.09x104 in Kpakungu. There was no coliform growth on the aseptically prepared bean cakes and those from Bosso and Dutsen kura.

In this study the following organisms were isolated: *Bacillus subtilis, Micrococcus roseus, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Aspergillus niger. Staphylococcus aureus* had the highest frequency of occurrence while *Aspergillus niger* had the least. This could be due to the fact that *S. aureus* are widely distributed in the environment and occur on the skin and nostrils ofhumans (Adele *et al.,* 2018)**.** It is thereforepossible that these organisms can be transferred from these sources to the bean cakes during processing.

*Aspergillus niger*was also isolated. These organisms are widely distributed in the environment and produced spores which are heat resistant and allow them to proliferate in the Akara. Makun (2007) reported that *Aspergillus* species contaminates peanuts and other foods producing aflatoxins, a potent carcinogen. This is similar to the findings of Bala *et al.* (2012) where species of *bacillus, Staphylococcus* and *Aspergillus* were isolated from stages four (fried bean cake) and five (storage) of bean cake production respectively. The findings are also similar to those of the reports for the occurrence of these organisms in a wide variety of food where they cause food infection, poisoning and intoxication (Adele *et al.,* 2018)**.** The isolation of these microbes from bean cakes is worrisome because these organisms are potential pathogens of man capable of causing a variety of diseases.

Generally in this study, the bean cakes collected from the sale points had high microbial growth while the aseptically prepared bean cakes yielded no growth. This could be due to the mode of handling of the bean cakes and the level of hygiene of the handlers and the environment. This is in line with the findings of Toledo *et al.* (2013) who observed that aseptic processing can eliminate spoilage microorganisms.

The molecular identification of the isolates confirmed the isolates as *Bacillus subtilis, Micrococcus roseus, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Aspergillu sniger.*  This is similar to the findings of Mpinda *et al.* (2018). Who identified these organisms from street -vended ready -to -eat meat.In this study, the antimicrobial susceptibility profile shows that *Staphylococcus aureus*, *Klebsiella pneumonia* and *Micrococcus roseus* were susceptible to 45.45% of the antibiotics used while *Escherichia coli* was susceptible to 31.98%, *Bacillus subtilis* was susceptible to 27.27% and *Aspergillus niger* was susceptible to 60% of the antifungal agents used. This is similar to the findings of Alum *et al.* (2016) where *Staphylococcus aureus* and *Klebsiella pneumonia* were susceptible to 47% of the agents used

**CONCLUSION**

*Aspergillus niger, Bacillus subtilis, Micrococcus roseus, Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli* were isolated and characterized from bean cake sold in Minna metropolis. The microbial load of the bean cake samples collected were higher than the aseptically produced one. The study revealed the critical control points of the production of Akara from bean paste production (milling) and water used for mixing. These are points where some degree of control can be exercised over the microbial hazard to prevent, eliminate or reduce the microbial level to acceptable limits. The isolation of these organisms is a serious public health concern. Their presence is associated with poor environmental hygiene.

**RECOMMENDATIONS**

Based on the findings of this research, it is recommended that:

1. thorough washing and mixing of the bean cakes with clean water should be undertaken to reduce the level of microbial contamination.
2. Aseptic techniques are essential in the sales point.
3. Contamination of the bean cakes by bare hands can be reduced by hand washing and good personal hygiene. Avoid insect contamination by covering the cakes after frying.

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