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Microbiological Assessment and Diversity of Freshly Harvested and Stored Peanuts (*Arachis hypogaea*): Implications for Food Safety and Shelf Life

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Peanuts (*Arachis hypogaea*) are a vital and affordable source of nutrients, providing essential proteins, lipids, vitamins, and minerals. However, their susceptibility to microbial contamination poses significant food safety and shelf-life concerns. This study aimed to assess the microbiological quality and diversity of freshly harvested and stored peanuts, with implications for food safety and shelf life. Microbial analyses revealed that freshly harvested peanut seeds sampled from local markets in Obio-Akpor, Rivers State, Nigeria exhibited higher bacterial and fungal loads compared to stored samples, with viable bacterial counts of 5.4×10^4 and 7.2×10^4 cfu/g for freshly harvested seeds, and 3.2×10^3 and 3.35×10^3 cfu/g for stored seeds. Fungal counts followed a similar trend, with higher loads observed in freshly harvested samples (3.6×10^3 and 4.0×10^3 cfu/g) compared to stored seeds (1.2×10^3 and 1.8×10^3 cfu/g). The higher microbial load in freshly harvested peanuts was attributed to the lack of preprocessing, while the lower counts in stored samples were linked to industrial processing steps, including drying and salting, which reduce moisture content and inhibit microbial proliferation. Microbial diversity analysis identified *Bacillus sp.*, *Micrococcus sp.*, *Staphylococcus sp.*, and *Proteus sp.* in both fresh and stored samples, with *Serratia sp.* isolated exclusively from freshly harvested peanuts and *Enterobacter sp.* found only in stored samples. Among fungal species, *Aspergillus niger* and *Aspergillus flavus* were predominant in both sample types, while *Fusarium sp.*, *Alternaria sp.*, and *Trichoderma sp.* were isolated solely from freshly harvested peanuts. Shelf-life studies demonstrated a progressive increase in microbial load over time, with higher rates observed in freshly harvested peanuts. The antibiotic susceptibility profile indicated resistance to Nalidixic acid, cephalexin, and penicillin among Gram-positive isolates, with high sensitivity to Ciprofloxacin, Ofloxacin, and Clindamycin. Gram-negative isolates were generally susceptible to the tested antibiotics, except for Norfloxacin, which was ineffective against *Proteus sp.* and *Serratia sp.* from freshly harvested samples. This study underscores the importance of preprocessing, such as salting and drying, to extend the shelf life of peanuts and mitigate microbial spoilage. The findings highlight the critical role of moisture control in reducing microbial contamination, thereby enhancing food safety and extending the shelf life of peanuts. Recommendations include the adoption of preprocessing techniques for subsistence farmers and the need for timely consumption of freshly harvested peanuts to minimize microbial deterioration.

Keywords: Peanut (Arachis hypogaea); microbial spoilage; contamination; fungal; bacterial; food safety; microbiology; pathogens.

1. INTRODUCTION

“Peanuts (*Arachis hypogaea*) are one of the most important oilseed crops and snack foods in the world Agro-food trade market” [1]. “The major producers/exporters of peanuts are the United States, China, Argentina, Sudan, Senegal, and Brazil. Peanuts are globally used as an economical food source as they contain high-quality proteins, and unsaturated fatty acids, and are rich in minerals. Peanuts are consumed whole roasted and boiled, and as ingredients in variety forms of products including peanut brittle, peanut butter, peanut oil, and so on” [2]. It was introduced into Nigeria in the 16th century and it has been estimated that about 1.5 to 2 million hectares are cultivated for peanuts in Nigeria [3,4].

“In Nigeria, the areas where peanut is mainly grown in the northern part of Nigeria; over 85% of the groundnuts produced in the country were accounted for by Kano, Kaduna, Taraba, Bauchi, Bornu, and Adamawa states” [5]. “Recently, the use of groundnut meal is becoming more recognized not only as a dietary supplement for children on protein-poor cereals-based diets but also as effective treatment for children with protein related malnutrition. It is the 13th most important food crop of the world and the 4th most important source of edible oil” [5].

“Peanut has many importance; peanut sauce, prepared with onions, garlic, peanut butter/paste, and vegetables such as carrots, cabbage, and cauliflower, can be vegetarian (the peanuts supplying ample protein) or prepared with meat, usually chicken” [5]. “Peanuts are used in the Mali meat stew “maafe”. In Ghana, peanut butter is used for peanut butter soup nkate nkwan. Crushed peanuts may also be used for peanut candies nkate cake and kuli-kuli, as well as other local foods such as oto. Peanut butter is also an ingredient in Nigeria’s “African salad”. Peanut

powder is an important ingredient in the spicy coating of kebabs in Nigeria and Ghana" [5]. "Peanuts are also used in a wide variety of other areas, such as biodiesel fuel, peanut laxatives, peanut dye, peanut shampoo, peanut insecticide, peanut explosives, peanut glue, etc" [5].

Peanuts can be consumed both raw or roasted in Nigeria. Seeds are roasted after the outer shell is not broken or the shell is broken and it is presented for consumption. Peanuts can be sold as packaged and unpackaged in snack shops at room temperature. It is sold also raw shelled peanuts on the street. These conditions make the peanut vulnerable to the microbiological risks.

Peanuts are rich in energy (567 calories per 100 g) and contain health-benefiting nutrients, minerals, antioxidants, and vitamins that are essential for optimum health. They compose sufficient levels of monounsaturated fatty acids (MUFA), especially oleic acid. MUFA helps lower LDL or "bad cholesterol" and increases HDL or "good cholesterol" levels in the blood. Although a legume; it is generally included amongst the oilseeds due to its high oil content. "Peanuts are rich in protein, oil, and fibers" [6]. Research studies suggest that the Mediterranean diet which is rich in monounsaturated fatty acids helps prevent coronary artery disease and stroke risk by favoring a healthy serum lipid profile. Peanut kernels are a good source of dietary protein; composed of fine quality amino acids that are essential for growth and development. Research studies have shown that peanuts contain high concentrations of polyphenolic antioxidants, primarily p-coumaric acid.

This compound has been thought to reduce the risk of stomach cancer by limiting the formation of carcinogenic nitrosamines in the stomach. Peanuts are an excellent source of resveratrol, another polyphenolic antioxidant. Resveratrol has been found to have a protective function against cancers, heart disease, degenerative nerve disease, Alzheimer's disease, and viral/fungal infections. Furthermore, studies suggest that resveratrol may reduce stroke risk by altering molecular mechanisms in the blood vessels (reducing susceptibility to vascular damage through decreased activity of angiotensin, a systemic hormone responsible for blood vessel constriction that would elevate blood pressure), and by increasing the production of vasodilator hormone, nitric oxide. Recent research studies suggest that roasting/boiling enhances antioxidant bioavailability in peanuts. It has been found that boiled peanuts have two and four-fold increases in isoflavone antioxidants biochanin-A and genistein content, respectively. Peanut fat profile contains about 50 % monounsaturated fatty acids (MUFAs), 33 % Paraformaldehyde (PFAs), and 14 % saturated fatty acids which is a heart-friendly combination of fatty acids (*Oke & Adetunji, 2021*).

"The kernels are an excellent source of vitamin E (α -tocopherol); containing about 8 g per 100 g. Vitamin E is a powerful lipid-soluble antioxidant that helps maintain the integrity of mucosa and skin by protecting it from harmful oxygen free radicals. The nuts are packed with many important B-complex groups of vitamins such as riboflavin, niacin, thiamin, pantothenic acid, vitamin B-6, and folates. 100 g of peanuts provide about 85% of the RDI of niacin, which contributes to health and blood flow to the brain. The nuts are a rich source of minerals like copper, manganese, potassium, calcium, iron, magnesium, zinc, and selenium. Just a handful of peanuts a day provide enough recommended levels of phenolic antioxidants, minerals, vitamins, and protein. Peanuts (*Arachis hypogea*) are commonly used to produce either candies or salted snacks. The commercial price of this commodity is determined by its aflatoxin content, since this mycotoxin is associated with liver cancer, central nervous system damage, hormonal disturbances, and even death, depending on the dosage" [7].

"During the primary production of peanuts, environmental conditions (temperature, rainfall, and humidity) contaminated soil, contaminated irrigation water, inadequate environmental hygiene, the presence of animals and mechanical damage caused by insects could influence the load and kind of microbiological contamination of peanuts from the harvesting period to post-harvesting" [2]. "The initial microbiota of peanuts which develop beneath the soil surface originates from the soil. Some of the Aspergillus species are ubiquitous invaders of nuts and can produce mycotoxin aflatoxin. Microbial contamination of peanuts occurs while the grain is growing in the field, these contaminants can increase while the grains are actively growing and also after harvesting" [2]. Mycotoxins are toxic compounds produced as metabolic products by fungi that could cause physiological or pathological changes in man or animals. The toxicity syndromes resulting from

the intake of such contaminated animals and man usually by ingestion have been termed mycotoxicosis. These are distinguished from mycoses which involve a generalized invasion of living tissues by actively growing moulds. Interest in these products resulted from the discovery in Great Britain that they were found to be produced by some *Aspergillus species*.

"So far although there have not been studies on the microbiological characteristics of the peanut, which is thought to be clean from a microbiological point of view, since 1960, studies only on aflatoxin, a mycotoxin that the molds have produced, have been extensively carried out. However, almonds, cashews, coconut, hazelnuts, pine nuts, pecans, pistachios, sesame seeds, and peanuts, as well as several processed nut products, have been associated with foodborne outbreaks and/or recalls after isolation of foodborne pathogens. It was seen that the majority of these outbreaks and recalls have been related to *Salmonella,* and many of the outbreaks have lasted for months and have included cases from multiple states in the United States and/or from other countries. Therefore, most published surveys have focused on indicator organisms and *Salmonella*. In the United Kingdom, the incidence of *Salmonella* on retail edible nuts and seeds was under 1%; samples included roasted nut kernels (25 g, n~727, 11 nut species, processed (heat or added preservatives) nuts (25 g, n~2,886, 12 nut species) and dried seeds (25 g, n ~ 3,735). The prevalence of *Salmonella* on raw almond kernels was about 1% over 8 years (100 g, n ~ 13,972)" [8]. "Outbreaks and recalls due to *Escherichia* coli O157:H7 and recalls due to *Listeria monocytogenes* contamination also are documented for some nuts and nut products" [9]. "Low aw foods have usually been considered safe regarding foodborne pathogens because the optimum aw for the growth of these pathogens is over 0.95. But unfortunately, there have been several outbreaks were seen also due to foods which were low water activity contaminated with *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes"* [10]. "Dry foods like peanuts do not often support microbial growth, but they may still be able to allow survival of pathogens. *Komitopoulou and Penaloza (2009)* found that inoculated Salmonella could survive for 3-4 weeks on dry, raw materials including crushed cocoa and hazelnut shells, cocoa beans, and almond kernels at both room temperature and 5°C. Salmonella is typically inhibited by aw<0.91, but the aw of these substances was likely much lower" [11]. "Pathogens can be transferred in several ways to food, such as through contaminated water and equipment, poor worker hygiene, and pests" [11]. "At harvest, if good agricultural and hygiene practices are followed, nuts are harvested with minimal damage and then dried to a water activity of less than 0.7. The Codex International Code of Hygienic Practice for tree nuts states that these products should be free from pathogenic microorganisms. Although nut-associated outbreaks of infection are relatively uncommon, recent outbreaks of salmonellosis associated with the consumption of peanuts, peanut products, and almonds have raised awareness of nuts as a potential vehicle for foodborne illness" [12]. For this reason, this study was designed to evaluate the microbiological quality of the peanuts produced in Nigeria. This study was the first investigation the microbiological quality of peanuts in our country and peanuts were evaluated for pathogens such as *Salmonella*, *Listeria spp.*, Coliform and *E. coli*, as well as mold, yeast, and total aerobic mesophilic bacteria.

2. MATERIALS AND METHODS

2.1 Study Location

Samples were purchased from a wholesaler at the local market in Obio-Akpor Local Government area in Rivers State.

2.2 Collection of Samples

Three (3) cups of locally produced peanut (freshly harvested) and three (3) cups of stored peanuts of the same species with a weight of 160grams each were purchased from a wholesaler at the local market in Obio-Akpor Local Government area in Rivers State after which the mass of 10grams were removed from the mass of 160grams for the analysis and the rest kept for shelf-life microbial studies. The samples were put into sterile containers and sent to the lab for further analysis. Important safety sampling techniques were observed to avoid contaminating samples or exposing themselves to contaminants; items such as gloves and protective clothing, use of sterile containers, and equipment were used. Containers were labeled accurately noting; the date and time of collection, description of what was sampled, and sampling site. Name of the person who collected it, CCP in this step involves wearing protective equipment during collation to avoid contamination.

2.3 Sterilization

Glasswares were washed properly in clean water and allowed to dry. Instruments like inoculation loops were sterilized with red heat from a Bunsen burner flame. Spreader or hockey Sticks were sterilized by dipping them first into 70% alcohol and igniting them in the Bunsen flame. Sterile disposable was used throughout the experiment.

2.4 Media Preparation

The following media were employed during this study. All media was prepared according to the manufacturer's specifications.

2.5 Nutrient Agar

Twenty-eight (28) grams of nutrient agar (NA) powder were weighed on an electrical weighing balance and transferred into a conical flask containing 1000ml distilled water. The mouth of the conical flask was plugged with non-absorbent cotton wool and then wrapped with aluminum foil. The mixture was swirled and heated to obtain a homogenized solution. The solution was autoclaved at 120ºC for 15 minutes, after which the prepared medium was allowed to cool down to about 45˚C, and then poured into Petri dishes and allowed to solidify.

2.6 Potato Dextrose Agar

The potato dextrose agar was prepared according to the manufacturer's instructions; 39g of powdered potato dextrose agar (P.D.A. BHEWADI-301 019) was weighed on a weighing balance and transferred into a 1000ml conical flask containing 500ml of Distilled water after which the suspension was stirred to dissolve the solid and heated to homogenize. After heating the mouths of the conical flask were wrapped with aluminum foil followed by sterilization of the medium in an autoclave 121psi for 15mins and then allowed to cool to about 45 degrees Celsius. Lactic acid 0.1% was added to prevent bacterial growth in the medium. The plates were poured and allowed to set.

2.7 Nutrient Broth

Fifteen (15) grams of nutrient broth powder were added to 1L of distilled water, mixed, and dissolved completely after which it was sterilized by autoclaving at 121°C for 15 minutes.

2.8 Bacteria Enumeration Using Standard Plate Count

The growth of bacteria was estimated using the standard plate count. In the standard plate count, a sample of bacteria is diluted and then plated on Nutrient Agar and then the plates are incubated overnight thereby leading to the formation of colonies. The colonies were counted visually and then the total number of bacteria would be calculated from the dilution factor and the volume plated on the media.

cfu/ml = (no. of colonies x dilution factor) × inverse of volume of culture plated

2.9 Identification and Characterization of Isolates

Single colonies of bacteria growth on plat count agar were randomly selected from different media plates based on their morphology and were subculture and incubated at 37 ˚C for 24 hours (h) to obtain pure colonies.

2.10 Examination of Bacteria

Isolates were identified based on their morphological and cultural characteristics on growth media. Identification materials, reagents, and protocols according to Cheesebrough, (2000) and Bergey's Manual for Systematic Bacteriology 2nd ed (Volume 3 for example is on Firmicutes/Gram-positive bacteria) were used to identify discrete colonies from the bacteriological media of sub-cultured isolates.

2.11 Gram Reaction of Bacteria

"Gram staining was carried out to differentiate between Gram-positive and negative organisms. A sterile wire loop was used to make a smear on the glass slide by taking a drop of distilled water on the slide, the wire loop was sterilized again by flaming and inoculums were picked, placed on the slide, and heat-fixed by passing the slide over the Bunsen burner flame. The slide was flooded with crystal violet for 60 sec and rinsed with water to wash unbound dye. Gram's iodine was added for another 60 sec and rinsed with water after which (95% alcohol) was added for 30 sec to remove unbound dye and immediately rinsed with water followed by the addition of safranin for counterstaining for another 30 sec and rinsed with water. Excess stain was wiped off from the slide using cotton wool. The slide

was air-dried and ready for microscopic examination (oil immersion of x 100)” [13].

2.12 Biochemical Characterization of Bacteria Isolates

“The identities of the isolates were confirmed using biochemical tests” (Cheesebrough, 2005). Tests carried out include indole, catalase, methyl red production, Voges-Proskauer reaction, citrate and triple sugar iron agar test (TSIA), and sugar fermentation tests for all isolates.

2.13 Catalase Test

“This test was done to differentiate between bacteria that produce the enzyme from non-catalase-producing bacteria. A drop of distilled water was placed on a clean glass slide after and a colony was picked using a sterile inoculating loop and emulsified with water and a drop of hydrogen peroxide. The production of bubbles is an indication that oxygen was given off which indicates a positive result” [13].

2.14 Indole Production

“The test was used to determine the ability of certain microorganisms to break down the amino acid tryptophan in the medium into indole in the presence of the enzyme tryptophanase. The test organism was inoculated into test tubes containing 10 ml of sterile tryptone broth and incubated for at least 48 h at 35-37 ˚C. After which 0.5 ml of Kovac’s reagent was added to the media and shaken gently and examined for a red color in the surface layer which indicates a positive result and no color change indicates a negative result” [13].

2.15 Citrate Utilization

This test was used to determine if an organism can utilize citrate as its sole source of carbon and energy. The citrate test uses a medium in which sodium citrate is the source of carbon and energy. Slants of Simon’s citrate agar were prepared in bijou bottles and the test organisms were inoculated by streaking the surface and stabbing the butt with a sterilized inoculating needle and incubated at 35 ˚C for 48 h and were observed for a bright blue color in the medium which indicates a positive result.

2.16 Motility Test

The motility test was done to determine if the organism was motile or not motile by moving away from the line of inoculation. A sterile wire loop was used to pick an isolate, stabbed directly into the center of the test tubes containing the motility agar, and incubated for 18-24h at 37 ˚C. A positive result shows a diffused growth away from the line of inoculation and no diffused growth indicates a negative result.

2.17 Methyl Red (MR) and VogesProskauer (VP) Tests

This is made up of two tests; methyl red and Voges-proskauer test. The methyl red test was used to determine if any organisms performed mixed acid fermentation in glucose-buffered broth. The methyl red indicates the production of sufficient acidic products from the fermentation of glucose while the Voges-Proskauer test indicates the production of acetoin from the fermentation of glucose. The MR-VP broth (10 ml) was inoculated with the test organisms and incubated for 48 hours. After incubation, 5 ml of the test culture was transferred aseptically to a clean test tube for the VP test. 3-4 drops of methyl red were added to the first test tube. A positive reaction is indicated by a distinct red color showing the presence of acid. A yellow color indicates a negative result.

For the Voges- Proskauer test, 0.6 ml of alpha-naphthol and 0.2 ml of 40 % potassium hydroxide were added to the second test tube. The broth was left to stand for 15 min for colour development after thorough agitation. If acetoin was produced, there would be a red color change. A yellow to brown color indicates a negative result.

2.18 Oxidase Test

The test was used to determine the presence of cytochrome oxidase. Kovac’s oxidase reagent is turned purple by organisms containing cytochrome C as part of their respiratory chain.

A few drops of Kovac’s oxidase reagent were put on strips of filter paper and allowed to dry, sterile wire loop will be used to pick a loopful of the isolates from the culture media and streaked across the filter paper and will be observed for a color change.

2.19 Starch Hydrolysis Test

This test was carried out to ascertain if the organism is capable of utilizing glucose or starch as its sole source of carbon on a solid media

after incubation. The Petri plate containing the test organism was flooded with gram's iodine after incubation for 24 h at 37˚C and the presence or absence of a zone of clearance was observed.

2.20 Triple Sugar Iron Test

This test is carried out to identify organisms that can produce hydrogen sulphide (H2S). 6.5 g of triple sugar iron agar was dissolved in 1 L of distilled water and dispensed into test tubes in 10mls and plugged in with cotton wool. The test tubes were sterilized by autoclaving at 121oC for 15 min and slanted to solidify. The isolates were inoculated into sterile test tubes using an inoculating needle and streaked across the top of the slant and incubated for 24 h at 35 ˚C and were observed for gas production, acid, alkaline production, and hydrogen sulphide production.

2.21 Sugar Fermentation Tests

This test detects the ability of bacteria to ferment a particular sugar (glucose, sucrose, and lactose). One gram (1g) of each sugar powder was dissolved into 1 L of distilled water, 3 ml of Bromocresol purple was added into it allowed to soak for 10 min, and swirled to mix. 5 ml of each sugar was dispensed into test tubes and Durham's tube was placed inside inverted and plugged with cotton wool. The tubes were sterilized by autoclaving at 121 ˚C for 15 psi at 15 min. The isolate is inoculated into each test tube containing the sugars and incubated at 37 ˚C for 24-48 h. The development of yellow color indicates acid production and the presence of gas in Durham's tube indicates gas production.

2.22 Antibiotics Sensitivity Test

Antibiotic sensitivity patterns of all the confirmed Gram's positive and negative isolates were performed by standard disk diffusion method according to Kirby-Bauer on Mueller-Hinton agar (Titan, Biotech Ltd, Indian) following the procedures recommended by CLSI (2012). Commonly used antibiotics include Clindamycin (CN), Augmetin (AU), Ciprofloxacin (CPX), Sulfathiazole Trimethroprim (SXT), Streptomycin(S), Cephalexin (CEP), Ofloxacin (OFX), Nalidixic acid (NA), Prefloxacin (PEF), Erythromycin (E), Lerofloxacin (LEV), Ampiclox (APX), Rifampicin (RD), Amoxil (AML). A bacterial culture was prepared in sterile saline, from which 0.1mL was inoculated onto Mueller Hinton agar, after which antibiotic discs were carefully and aseptically placed on the surface of the agar. The plates were incubated at 37 ˚C for 24h. The zone of inhibition was measured in millimeters.

2.23 Examination of Fungi

Identification of fungi isolate were done according to Pictorial Atlas for Soil and Seed Fungi 3rd ed by Tsuneo Watanabe.

2.23.1 Isolation

Five (5) test tubes each containing 9ml distilled water were used for the dilution; 1g of a sample (sample A) was taken and transferred into the first test tube and allowed to dissolve, then mixed; to the second tube, 1ml of the content from the first test-tube is aseptically taken using a sterile syringe and poured into the second (2) test tube. This procedure was repeated up to the last test tube (5 test tubes). Thus, making a dilution factor of 101, 102, 103, 104, and 105. At the 5th test tube after shaking the solution 0.1ml was drawn and inoculated onto a Petri dish each containing prepared potato dextrose agar (PDA). They were covered and kept at room temperature of 25 ˚C -30 ˚C for 7 days. The serial dilution was done to deconcentrate the fungal content in the solution that may grow to fill the Petri dish. After one week of inoculation and incubation, plates were observed and found with different colors of fungal colonies, the record of several colonies was taken for frequency of occurrences. They were then sub-cultured for pure isolate. This same practice was carried out for samples B and C.

2.23.2 Subculture

Another Potato Dextrose Agar (PDA) was prepared and allowed to solidify. A loopful of the fungal culture was streaked unto the sterile PDA and incubated for seven (7) days at 250˚C-300˚C and thereafter fungal growth on the plate were observed and further analyzed microscopically.

3. RESULTS

3.1 Total Heterotrophic Bacteria Count of Peanut Samples Studied

The total heterotrophic bacterial counts revealed that the fresh Peanut samples (FGS 1 and FGS 2) studied had viable counts of 5.4×10^4 and 7.2×10^4 CFU/g respectively while the stored Peanut samples (SGS 1 and SGS 2) had counts

of $3.2x10^3$ and $3.35x10^3$ CFU/g respectively. The bacterial count ranged from an average count of 6.3×10^4 cfu/g of the two fresh groundnut seed Sample to an average of 3.27×10^3 cfu/g of the two stored groundnut seed samples. There is a difference of more than 3.0 $\times10^3$ cfu/g, this reduction in bacterial count is due to reduced or eliminated moisture content in the stored groundnut seed samples. Moisture(water) content which is necessary for microbial growth is eliminated or reduced by heating methods such as oven drying sun drying or salting, this technique is carried out before storage of groundnut seeds. Only a Dilution of 10^2 was used in the analysis of bacterial growth for the fresh Peanut seeds and a Dilution of 10^1 was used for the analysis of bacterial growth for stored Peanut samples this was because only dilutions that give a plate count of 30-300 colonies of bacteria is used. The total heterotrophic bacterial count of the peanuts sampled is shown in Table 1.

3.2 Total Fungal Count of Peanut Samples Studied

The fungal counts revealed that the fresh Peanut samples (FGS 1 and FGS 2) studied had fungal loads of $3.6x10^3$and $4.0x10^3$ CFU/ while the stored Peanut samples (SGS 1 and SGS 2) had loads of $1.2x10^3$ and $1.8x10^3$ CFU/g respectively. The fungal count ranged from an average count of 3.8×10^3 cfu/g of the two fresh groundnut seed Sample to an average of 1.5×10^3 of the two stored groundnut seed samples. The difference in the average fungal counts between the stored and fresh groundnut seeds is more than 2.0 $\times10^3$ cfu/g, this reduction in bacterial count is due to reduced or eliminated moisture content in the in the stored groundnut seed samples. Moisture(water) content which is necessary for microbial growth is eliminated or reduced by heating methods such as oven drying sun drying or salting, this technique is carried out before storage of groundnut seeds. Only a Dilution of 10^1 was used in the analysis of fungal growth for the fresh Peanut seeds and a Dilution of 10^1 was used for the analysis of fungal growth of stored Peanut samples this was because only dilutions that give plate count of 30-300 colonies of fungi is used. The total fungal count of the peanut seeds sampled is shown in Table 2.

3.3 Cultural Characteristics of Bacteria Isolated from Peanut Samples

The morphological characteristics of the isolates from the various Peanut samples studied are presented in Table 3. The cultural characteristics include; Colour (creamy, yellow, golden, red) when viewed unaided, Size of the colony (can vary from large colonies to tiny colonies less than 1mm), Edge/Margin of colony (magnified edge shape using a dissecting microscope to see the Margins well), Opacity of colony (how much the colony rose above the agar), Surface of colony (smooth, rough, dull, rugose, shiny), Shape (round or Irregular). The cultural characteristics of all isolates gotten were all detailed in the appropriate labels.

3.4 Gram Reaction/Biochemical Characteristics of Bacteria Isolated from Peanut Samples

Biochemical analysis of the individual isolates obtained from the different Peanut samples studied showed they harbored six (6) bacterial species including *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp., *Proteus* sp., *Serratia sp*., and *Enterobacter* sp. All the individual isolates tested positive for the catalase test apart from *Enterobacter* sp., this indicates that the organisms are obligate aerobes and were able to produce oxygen bubbles (*Facklam and Elliott, 1995*). Micrococcus sp. only was positive to oxidase test, this differentiates it as a positive pseudomonacae [14]. Proteus sp. Only was indole positive because of its ability to convert tryptophan Amino acids to indole gas, the gas reacted with the reagent and the color rosin-dole dye indicated a positive test [15]. In the TSIA test; proteus sp., Enterobacter sp. Where able to utilize, glucose-producing acid and gas causing a color change in both slant and butt's, *Bacillus* sp., *Staphylococcus* sp., *Proteus* sp., *Enterobacter* sp. are strict aerobes causing a color change only in the slant area. Production of hydrogen sulphide gas by the reduction of many species of bacteria is evidenced by the rising or breaking of agar medium [16]. *Proteus* sp. tested positive for the Methyl Red test, it was able to convert glucose to other forms of acids, by decreasing the pH of the medium and changing the color of the methyl red from yellow to red as an indicator that the organism has used up the glucose in the medium (*Crown and Gen, 1998*). All isolates apart from Enterobacter sp. were positive on the citrate test, the isolates were able to use citrate as their source of energy, and the change in pH changed the color of bromothymol blur from green to blue on the medium when the PH rose above 7.6. Bromothymol blue is used as an inductor in the CAU test (Jawetz et al. *1989*). The biochemical characteristics of the bacterial isolates are well detailed in Table 4.

Table 1. Total heterotrophic bacteria count of peanut samples studied

Sample Code	Dilution Plated	Av. Colony Count	CFU/g	Log CFU/g
FGS 1	10^{-2}	54	$5.4x10^4$	4.732
FGS 2	10^{-2}	72	$7.2x10^4$	4.857
SGS 1	10^{-1}	32	$3.2x10^3$	3.505
SGS 2	10^{-1}	33.5	$3.35x10^3$	3.525

Table 2. Total fungal count of peanut samples studied

Sample Code	Dilution Plated	Av. Colony Count	CFU/g	Log CFU/g
FGS 1	10^{-1}	36	$3.6x10^3$	3.556
FGS 2	10^{-1}	40	$4.0x10^3$	3.602
SGS 1	10^{-1}	12	$1.2x10^3$	3.079
SGS 2	10^{-1}	18	$1.8x10^3$	3.255

Table 3. Cultural characteristics of bacteria isolated from peanuts

Isolate code	Colour	Size (mm)	Shape	Margin	Opacity	Elevation	Surface	Texture
FGS 1A	Yellow	0.5	Round	Entire	Opaque	Convex	Shiny	Smooth
FGS 1B	Cream	2	Irregular	Lobate	Opaque	Mucoid	Dull	Smooth
FGS 1C	Cream	4	Round	Lobate	Opaque	Flat	Dull	Rough
FGS 2A	Golden	0.5	Round	Entire	Opaque	Flat	Shiny	Mucoid
FGS 2B	Cream	2.0	Round	Entire	Opaque	Raised	Dull	Smooth
FGS 2C	Cream	2	Irregular	Lobate	Opaque	Mucoid	Dull	Smooth
FGS 2D	Cream	2.0	Round	Entire	Opaque	Raised	Dull	Smooth
FGS 2E	Red	2	Round	entire	Opaque	Raised	shiny	Smooth
SGS 1A	Cream	2.0	Round	Entire	Opaque	Raised	SHINY	Smooth
SGS 1B	Yellow	0.5	Round	Entire	Opaque	Convex	Shiny	Smooth
SGS 2A	White	3	Round	Irregular	Opaque	Raised	Dull	Smooth
SGS 2B	Cream	4	Round	Lobate	Opaque	Flat	Dull	Rough
SGS 2C	brown	2.0	Round	Entire	Opaque	Flat	Shiny	Smooth

3.5 Cultural/Microscopic Characteristics of Fungi Isolated from Peanut

The macroscopic (cultural) and microscopic examination of the fungi isolated from all the Peanut samples studied showed that five (5) fungal genera including *Aspergillus flavus, Aspergillus niger, Fusarium* sp., *Alternaria* sp., and *Trichoderma* sp. were predominant as detailed in Table 5.

3.6 Frequency of Bacterial Isolates from Peanut Samples Studied

The frequency of bacteria isolated showed that 23.07% were *Bacillus*, 23.07% *Micrococcus* and 23.07% were *Staphylococcus*. 15.38 % were *Proteus;* 7.69% were *Enterobacter* and 7.69% *Serrattia* sp (Table 6).

3.7 Frequency of Fungal Isolates from Peanut Samples Studied

The frequency of fungi isolated showed 37.5% were *Aspergillus flavus; 25*% were A*spergillus niger*. *Fusarium, Alternaeria,* and *Tricoderma* species each represented 12.5% of the total fungal isolates (Table 7).

3.8 Antimicrobial Susceptibility Pattern of Gram-Positive Bacteria from Peanut

The antibiotics sensitivity pattern showed that all the Gram's positive isolates were resistant to Cephalexin (CEF) and Nalidixic acid (NA). Ciprofloxacin (CPX), Clindamycin (CN), Prefloxacin (PEF), and Ofloxacin (OFL) were all potent against all the Gram's negative bacteria isolated as they were all sensitive to them, according to Kirby-Bauer a diameter of 0-14mm is said to be resistant while 15mm- 17mm is intermediate from 18mm above is sensitive (Table 8).

3.9 Antimicrobial Susceptibility Pattern of Gram-Negative Bacteria from Peanut

Augmentin, ciprofloxacin, Erythromycin, Erythromycin (30mg), and lerofloxacin all showed potency against the Gram's negative isolates

from the Peanut samples studied. However, ampiclox showed intermediate sensitivity against Serratia sp. FGS2D and FGS2E were resistant to Norfloxacin but SGS2B was sensitive to it, (Table 9).

3.10 Bacterial Load During Shelf-Life Study of Peanut Spoilage

The bacteria load during the shelf-life study differed on the days investigated. FGS 1 and FGS 2 had counts of 5.4×10^4 and 7.2×10^4 CFU/g on day 0 while SGS1 and SGS 2 had loads of 3.2×10^3 and 3.35×10^3 CFU/g. Counts increased as days increased with the 15th day having counts of 1.25×10^5 and 1.56×10^5 for fresh samples and 1.0×10^4 and 1.24×10^4 cfu/g for the stored samples (Table 10).

3.11 Fungal Load During Shelf-Life Study of Peanut Spoilage

The fungal load during the shelf-life study was different on the different days investigated. FGS 1 and FGS 2 had counts of 1.5×10^3 and 2.0×10^3 CFU/g on day 0 while SGS1 and SGS 2 had loads of 5.0×10^2 and 9.0×10^2CFU/g. Counts increased as days increased with the 15th day having counts of 5.2×10^3 and 6.4×10^3 for fresh samples and 1.9×10^3 and 2.45×10^3 cfu/g for the stored samples (Table 11).

4. DISCUSSION CONCLUSION AND RECOMMENDATION

4.1 Discussion

This study aimed to assess the microbial quality of freshly harvested versus stored peanuts in Obio-Akpor Local Government Area, Rivers State, by analyzing bacterial and fungal contamination. The findings reveal significant differences in microbial loads between fresh and stored peanut samples, which are critical for understanding their shelf-life and safety.

The observed total heterotrophic bacterial counts of fresh peanuts (5.4×10^4 and 7.2×10^4 CFU/g) were significantly higher than those of stored peanuts (3.2×10^3 and 3.35×10^3 CFU/g). This reduction in bacterial load for stored peanuts aligns with findings by *Akinmoladun* et al. [17] who reported that proper storage conditions, including reduced moisture content, effectively inhibit bacterial growth in nuts. The reduction in bacterial counts in stored peanuts is attributed to the lower moisture levels, which are known to limit microbial proliferation [18]. Moisture reduction, achieved through methods such as sun drying or oven drying, decreases the water activity in peanuts, making the environment less conducive for bacterial growth [19].

Similarly, the fungal counts showed a decrease in stored peanuts (1.2×10^3 and 1.8×10^3 CFU/g) compared to fresh peanuts (3.6×10^3 and 4.0×10^3 CFU/g). This finding is consistent with the research by *Amadi* et al. [20] who observed that fungal contamination in stored grains significantly reduced due to lower moisture levels. This reduction in fungal load can be attributed to the same principle; moisture is a critical factor for fungal growth, and its reduction inhibits fungal proliferation [21].

The observed differences in microbial counts between fresh and stored peanuts underscore the effectiveness of moisture control in extending shelf life and maintaining the microbiological quality of peanuts. This is in agreement with findings by *Aslam* et al. [22] who emphasized the importance of controlling environmental factors, such as moisture, to reduce microbial contamination in stored food products.

The microbial isolates from the peanut samples were characterized using various biochemical tests, including Gram staining, catalase, indole production, citrate utilization, motility, and the MR-VP tests. The identification of bacterial isolates through these tests is supported by methodologies outlined in *Cheesbrough* [23] and Bergey’s Manual for Systematic Bacteriology [24].

The Gram staining results indicated the presence of both Gram-positive and Gram-negative bacteria, reflecting a diverse microbial population. This finding is consistent with research by *Ghosh* et al. [25] who reported a similar diversity of bacterial isolates in peanuts, with both Gram-positive and Gram-negative bacteria being commonly found. The use of Gram staining as a primary differentiation tool is well-documented and provides valuable insights into the types of bacteria present [26].

The catalase test, used to differentiate between catalase-producing and non-catalase-producing bacteria, revealed the presence of both types of bacteria. This test is crucial for identifying genera such as Staphylococcus and Micrococcus, which produce catalase [27]. Similarly, the indole production test was used to determine the ability of bacteria to degrade tryptophan into indole, which is a key characteristic for identifying certain Enterobacteriaceae [28].

Table 4. Gram reaction/biochemical characteristics of bacteria isolated from peanut samples

Isolate code	Gram Rxn Reaction	Cell morphology	Citrate	Catalase	Oxidase	Indole	Motility	MR	VP	Lactose	Glucose	Sucrose	TSIA				Tentative bacteria genera
													Butt	Slant	H_2S	Gas	
FGS 1A	+	Rod	+	+	-	-	+	-	+	A	A	-	B	A	-	-	*Bacillus* sp.
FGS 1B	+	Cocci	+	+	+	-	-	-	-	A	A	-	B	B	-	-	*Micrococcus* sp.
FGS 1C	+	Cocci	+	+	-	-	-	-	+	A	A	-	B	A	-	-	*Staphylococcus* sp
FGS 2A	+	Rod	+	+	-	-	+	-	+	A	A	-	B	A	-	-	*Bacillus* sp.
FGS 2B	+	Cocci	+	+	+	-	-	-	-	A	A	-	B	B	-	-	*Micrococcus* sp.
FGS 2C	+	Cocci	+	+	-	-	-	-	+	A	A/G	-	B	A	-	-	*Staphylococcus* sp.
FGS 2D	-	Rod	+	+	-	+	+	+	-	-	A/G	A	A	A	+	+	*Proteus* sp.
FGS 2E	-	Rod	+	+	-	-	+	-	+	-	A	-	A	B	-	-	*Serratia* sp.
SGS 1A	+	Cocci	+	+	-	-	-	-	+	A	A	-	B	A	-	-	*Staphylococcus* sp
SGS 1B	+	Cocci	+	+	+	-	-	-	-	A	A	-	B	B	-	-	*Micrococcus* sp.
SGS 2A	+	Rod	-	-	-	-	-	-	-	A/G	-	A/G	A	A	-	+	*Enterobacter* sp.
SGS 2B	-	Rod	+	+	-	+	+	+	-	-	A/G	A	A	A	+	+	*Proteus* sp.
SGS 2C	+	Rod	+	+	-	-	+	-	+	A	A	-	B	A	-	-	*Bacillus* sp.

Table 5. Cultural/microscopic characteristics of fungi isolated from peanut

Isolate code	Cultural characteristics	Cell morphology	Tentative genera of fungi
FGS 1A	Green-yellow velvety hayphae with creamy surrounding	Septate hyphae with conidia arranged like a mop head	*Aspergillus flavus*
FGS 1B	Whitish to porkish cotton-like surface with smooth reveres end	Septate hyphae which produce phalides from the branched conidia	*Fusarium* sp.
FGS 1C	Wody colonies with arranged blue or yellow-green patches with whitye surrounding	Septate hyphae with separate chlamydiospores from conidia	*Trichoderma* sp.
FGS 2A	Brown-black velvety hyphae with surrounding having cracked reverse	Septate phialides with conidia aerranged with mop-like appearance	*Aspergillus niger*
FGS 2B	White mycrlium with cream zones cracked reverse	thick-long unbranched hypha, with green spores	*Alternaria* sp
SGS 1A	Green-yellow velvety hayphae with creamy surrounding	Septate hyphae with conidia arranged like a mop head	*Aspergillus flavus*
SGS 2A	Brown-black velvety hyphae with surrounding having cracked reverse	Septate phialides with conidia aerranged with mop-like appearance	*Aspergillus niger*
SGS 2B	Green-yellow velvety hayphae with creamy surrounding	Septate hyphae with conidia arranged like a mop head	*Aspergillus flavus*

Table 6. Frequency of bacterial isolates from peanut samples studied

Isolate	Frequency
Bacillus sp.	3 (23.07%)
Micrococcus sp.	3 (23.07%)
Staphylococcus sp.	3 (23.07%)
Proteus sp.	2 (15.38%)
Enterobacter sp.	1 (7.69%)
Serratia sp.	1 (7.69%)
Total	13 (100%)

Table 7. Frequency of fungal isolates from peanut samples studied

Isolate	Frequency
Aspergillus flavus	3 (37.5%)
Aspergillus niger	2 (25.0%)
Fusarium sp.	1 (12.5%)
Altenaria sp.	1 (12.5%)
Trichoderma sp..	1 (12.5%)
Total	8 (100%)

Table 8. Antimicrobial susceptibility pattern of gram-positive bacteria from peanut

	Antibiotic									
Isolate	**CN**	**AU**	**CPX**	**SXT**	**S**	**PN**	**CEP**	**OFX**	**NA**	**PEF**
FSG 1A	S	S	HS	HS	S	R	R	S	R	HS
FSG 1B	S	R	HS	HS	H	R	R	HS	R	HS
FSG 1C	S	R	HS	I	S	R	R	HS	R	HS
FSG 2A	S	R	HS	HS	I	R	R	S	R	S
FSG 2B	HS	I	HS	HS	S	R	R	S	R	HS
FSG 2C	H	I	HS	R	HS	R	R	HS	R	HS
SGS 1A	HS	S	HS	R	S	R	R	S	R	S
SGS 1B	S	S	HS	HS	S	R	R	S	R	HS
SGS 2A	HS	I	HS	HS	S	R	R	S	R	HS
SGS 2C	S	S	HS	HS	S	R	R	S	R	HS

Key CN = Clindamycin (10mg), AU= Augmentin (30 mg), CPX = Ciprofloxacin (10mg), SXT = Sulfathiazole Trimethoprim (30mg), S= Streptomycin (30mg), CEP = Cephalexin (10mg), OFX = Ofloxacin (10mg), NA= Nalidixic acid(30mg), PEF= prefloxacin (10mg); HS= Highly Sensitive, S= Sensitive, R= Resistant.

Table 9. Antimicrobial susceptibility pattern of gram-negative bacteria from peanut

	Antibiotic									
Isolate	**AU**	**CPX**	**E**	**LEV**	**CN**	**APX**	**RD**	**AML**	**S**	**NB**
FGS 2D	S	S	S	S	S	I	S	I	I	R
FGS 2E	S	S	S	S	S	S	S	S	S	R
SGS 2B	S	S	S	S	S	S	S	S	S	S

Key: AU= Augmentin (30 mg); CPX = Ciprofloxacin (10mg); E= Erythromycin (30mg); E= Erythromycin (30mg); LEV= lerofloxacin (20mg); APX= Ampiclox (20mg); RD= Rifampicin (20mg); AML= Amoxil (20mg); NB = Norfloxacin (10mg)

Table 10. Bacterial load during shelf-life study of peanut spoilage

	Microbial load CFU/g			
Sample Code	**Day 0**	**Day 5**	**Day 10**	**Day 15**
FGS 1	$5.4x10^4$	$8.4x10^4$	$1.03x10^5$	$1.25x10^5$
FGS 2	$7.2x10^4$	$1.04x10^5$	$1.42x10^5$	$1.56x10^5$
SGS 1	$3.2x10^3$	$7.4x10^3$	$3.2x10^4$	$1.01x10^4$
SGS 2	$3.35x10^3$	$6.0x10^3$	$4.2x10^4$	$1.24x10^4$

Table 11. Fungal load during shelf-life study of peanut spoilage

	Fungal load CFU/g			
Sample code	**Day 0**	**Day 5**	**Day 10**	**Day 15**
FGS 1	$1.5x10^3$	$3.4x10^3$	$3.6x10^3$	$5.2x10^3$
FGS 2	$2.0x10^3$	$4.2x10^3$	$6.4x10^3$	$6.4x10^3$
SGS 1	$5.0x10^2$	$1.8x10^3$	$1.85x10^3$	$1.9x10^3$
SGS 2	$9.0x10^2$	$2.2x10^3$	$2.4x10^3$	$2.45x10^3$

Citrate utilization tests indicated whether bacteria could use citrate as the sole carbon source, with positive results suggesting the presence of bacteria such as Enterobacter and Klebsiella, which are known for their ability to utilize citrate [29]. The motility test helped identify motile bacteria, such as those in the genera Proteus and Pseudomonas, which are commonly associated with foodborne pathogens [30].

The MR and VP tests provided insights into the metabolic pathways utilized by the bacteria, with the MR test indicating mixed acid fermentation and the VP test detecting acetoin production. These tests are essential for differentiating between species of the Enterobacteriaceae family, as discussed by *Todar* [31].

The biochemical tests conducted in this study align with the methodologies recommended by

Bergey's Manual and other standard microbiological references, ensuring that the identification and characterization of bacterial isolates are robust and scientifically sound.

The antibiotic sensitivity test performed using the Kirby-Bauer disk diffusion method revealed the susceptibility patterns of the bacterial isolates to various antibiotics. This method, as outlined by the Clinical and Laboratory Standards Institute [32] is a well-established procedure for determining antibiotic resistance profiles.

The results showed varying degrees of resistance and susceptibility among the isolates, which is consistent with the findings of *Al-Bakri* et al. [33]. Variations in antibiotic resistance patterns can be attributed to the genetic and environmental factors affecting bacterial populations, as discussed by *Levy* et al. [34]. The observed resistance patterns highlight the need for continued monitoring of antibiotic resistance, particularly in foodborne pathogens, to ensure effective treatment and control measures.

The use of multiple antibiotics in the sensitivity test allows for a comprehensive assessment of bacterial resistance, providing valuable information for developing strategies to manage and mitigate antibiotic resistance in foodborne pathogens [35].

Fungal examination involved isolation and characterization of fungal colonies using Potato Dextrose Agar (PDA). The observed fungal colonies were identified based on their morphology and color, which is a standard approach for fungal identification [36]. The reduction in fungal counts in stored peanuts compared to fresh peanuts, as noted earlier, underscores the impact of storage conditions on fungal contamination levels.

The isolation and identification of fungi using PDA are supported by studies such as those by *Ellis* (2007) and *Samson* et al. [37] who have demonstrated the effectiveness of PDA in isolating a wide range of fungal species from food samples. The observed fungal species in this study are consistent with those reported in similar studies on peanuts [38].

5. CONCLUSION

This study provides a comprehensive analysis of the microbial quality of fresh and stored peanuts, highlighting the impact of moisture reduction on bacterial and fungal loads. The findings align with existing literature on the importance of proper storage conditions for maintaining food safety and quality. The identification and characterization of microbial isolates using biochemical tests and antibiotic sensitivity profiling offer valuable insights into the microbial composition and resistance patterns in peanuts.

Future research should explore the specific strains of bacteria and fungi present in peanuts and their potential impact on food safety. Additionally, investigating the mechanisms behind microbial resistance and developing effective control measures are crucial for ensuring the safety of peanut products.

6. RECOMMENDATIONS

1. **Improved Storage Practices:** Enhance storage conditions to include better moisture control and regular monitoring to further reduce microbial contamination.
2. **Regular Microbial Testing:** Implement routine microbial testing of both fresh and stored peanuts to ensure safety and quality, particularly focusing on antibiotic resistance patterns.
3. **Education and Training:** Educate local farmers and wholesalers about proper handling and storage techniques to minimize contamination risks and ensure food safety.
4. **Further Research:** Conduct additional studies to explore the specific types of fungi and bacteria prevalent in peanuts and their potential health impacts, which could inform more targeted interventions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX 1

Plate 1. Fungal isolates on pda plates

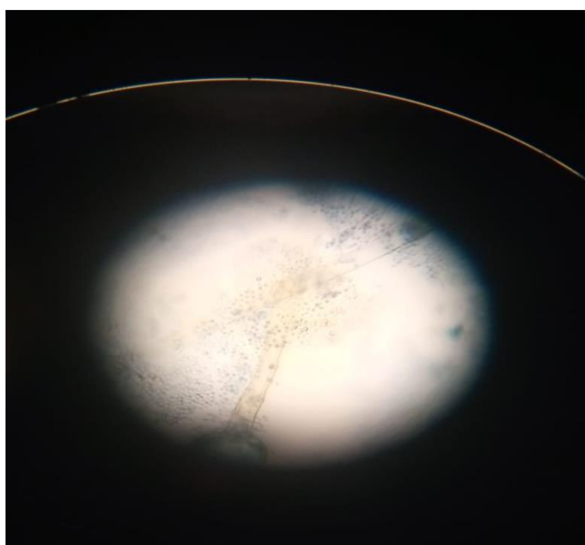

Plate 2. Fungi Isolates under a microscope (x40)

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