ISOLATION AND CHARACTERIZATION OF BACTERIA FROM FERMENTED CAYENNE PEPPER OBTAINED FROM MARKETS WITHIN ENUGU METROPOLIS

ABSTRACT

The study investigates the microbial diversity in fermented cayenne pepper sourced from four markets within Enugu metropolis. Fermented foods, including cayenne pepper, are notable for their unique flavors, nutritional benefits, and extended shelf life. This research aims to isolate and characterize the bacteria present in fermented cayenne pepper, identifying predominant species and evaluating variations in microbial composition across different markets. Samples were collected from Ogbete, Orie-Emene, Abakpa, and Obiagu markets, fermented for 21 days, and analyzed microbiologically using standard techniques. The study revealed diverse bacterial populations, including *Streptococcus lactis, Bacillus subtilis, Lactobacillus plantarum,* and *Enterococcus faecalis*. Fungal isolates identified were *Mucor spp., Aspergillus niger, Rhodotorulla spp.,* and *Geotricum spp.* Ogbete market exhibited the highest bacterial load (6.67 x 10^3 CFU/ml), while Abakpa had the least (1.68 x 10^3 CFU/ml). Bacterial occurrence was significantly higher across the market with frequency values above 79%. While fungi were below 21% in all cases. The study identifies the impact of environmental conditions and market practices on the microbial ecology of fermented foods, offering insights for optimizing food safety and quality control.

Keywords: Fermentation, Microbial diversity, Cayenne pepper, Market, Food safety.

INTRODUCTION

Fermented foods, including cayenne pepper, have been a significant part of human nutrition for decades due to their unique flavors, improved nutrition, and stable shelf lives. Cayenne peppers also known as chili pepper, chile pepper, hot pepper, and red chillies belongs to the *Capsicum* genus [1]. It has a rich history with increasing use by the day. Today, it is globally cultivated and utilized as a spice, condiment and vegetable across the globe. Its global production in 2017 was estimated as 36 million ton [2].

Cayenne pepper (*Capsicum annuum*) is majorly consumed for its culinary attributes worldwide as a spice included in many sauces and meals to impart pungent flavour and colour to dishes. Its pleasant colour aids consumer acceptance and value [3]. Cayenne pepper pungency is due to the availability of natural phenolic alkaloids groups known as capsaicinoids. These phytochemicals, vitamins, and fibers are effective in treating various health issues, however, cayenne pepper pungency limits its use [4]. The attributes of fresh cayenne peppers differ among varieties, environmental growth factors, cultivars, and maturity stages.

Hence, understanding fermented food composition is crucial to its quality and use [5]. Fermentation is a food processing method with the ability to reduce pungency through capsaicin degradation and enhance nutritional benefits in cayenne peppers. Spontaneous fermentation, a method used for vegetable preservation, involves bacteria breaking down complex nutrients into smaller, biologically assimilable substances [6]. Lactic Acid bacteria and yeasts, particularly *Lactobacillus* spp., produce exopolysaccharides, indicating beneficial probiotic activity.

This research focuses on exploring the microbial diversity of fermented cayenne pepper from four markets in Enugu Metropolis, by isolating and characterizing bacteria. To evaluate microbial composition, identify predominant species, and explore potential variations in composition.

Understanding these factors can have significant implications for food safety, quality control, and market regulations. Therefore, this study aims to isolate and characterize microorganisms from fermented cayenne pepper obtained from four markets within Enugu metropolis.

MATERIALS AND METHODS

Study Area and Sampling

The selected areas for this study are Ogbete market, Orie-Emene, Abakpa and Obiagu Market, all located within Enugu Metropolis. Cayenne pepper sample was randomly selected from each market in sterile containers. The analysis was carried out at Microbiology Laboratory of Godfrey Okoye university, and Panacea Diagnostic and Research Laboratory in Enugu.

Media, Chemicals and Reagent

The culture media used for this study includes Nutrient Agar, de Man, Rogosa and Sharpe Agar, Peptone Water Broth and Simmon's Citrate Agar. All were prepared according to standard as stated by the manufacturers. The reagent includes crystal violet, gram's iodine solution, ethanol, safranin, hydrogen peroxide reagent, distilled water, kovac reagent, methyl red reagent, oxidase reagent, normal saline and blood plasma.

Preparation of fermented Cayenne pepper

Fresh cayenne pepper (1kg) was sorted to remove the stalk and washed using distilled. The pepper was properly milled using a blender (3500 rpm). Milled samples were placed in transparent sterile

bottles, filled to the brim, the caps were pressed down to remove any air space and corked tightly. The samples were allowed to ferment for 21 days at 20 ± 1 ° [7]

Microbiological Analysis

Total microbial count

Nutrient agar medium was used for bacterial load and prepared by suspending, by adding 70g of nutrient agar in 1000ml of distilled water. Sabourand Dextrous Agar (SDA) medium was applied for fungi count and prepared by suspending 32.5g of SDA in 500 ml of distilled water. Each solution was boiled to dissolve separately, then sterilized in an autoclave for 15 minutes at 121°C, 15 psi and allowed to cool. Serial dilution was prepared using the fermented cayenne pepper stock solution. Then 9 ml of distilled water was measured and placed in each of the 9 test tubes i.e. serial dilutions of 10^{-1} , 10^{-2} , and 10^{-3} , were prepared in triplicates, in test tubes (all the tubes were autoclaved and allowed to cool before dispensing [8]. One ml of the fermented cayenne pepper solution was measured and dispensed into the 10^{-1} tube and was shaken, 1ml was transferred from 10^{-1} to 10^{-2} and was also shaken, then 1ml from 10^{-2} was transferred to the third test tube with concentration of 10^{-3}, in triplicates From the 10^{-3} dilutions, 1ml of the sample was dropped into a sterile petri dish, and the agar was poured into the petri dish enough to cover the base, using the pour plate technique. This was properly mixed and allowed to set and incubated at 37°C for 24 h for bacteria while fungi were incubated at 25°C for 72 h using Cinntex precision bacteriological incubator [9]. The colonies formed were counted and expressed in CFU/ml.

Pure cultures were obtained by sub-culturing on sterile fresh nutrient agar and SDA plates. The plates were incubated at 37°C for 24h and 25°C for 72 h, to identify the bacteria and fungi

respectively. The obtained pure culture was stored on agar slants and refrigerated at 4°C. Isolates were identified using morphological, biochemical and gram-staining tests [8,10].

Microscopic Test: Gram staining technique was utilized for microscopic testing, distinguishing between Gram-positive and Gram-negative groups by coloring cells red or violet.

A thin smear of each of the pure cultures was prepared on clean grease-free slides and stained for 30 seconds with crystal violet. The smear was then cleaned with distilled water. Gram's iodine was applied for 10 seconds, after which the smear was washed with water, decoloured with 95% acetone alcohol, and dyed with safranin for 30 seconds. The smear was then rinsed with tap water, dried by air, and examined with a 100X oil immersion objective [11].

Biochemical Test:

Biochemical tests identify bacterial species based on certain activities, including protein, fat, carbohydrate metabolism, enzyme production, and compound utilization ability, which are influenced by bacterial physiology differences [12].

Motility test:

This test was used to determine if the isolates were motile or non-mobile. The hanging drop technique was employed. Vaseline jelly was rubbed around the cavity of a hanging drop slide. A drop of peptone water containing the pure culture was placed on a cover slip. The hanging drops slide was then placed over the drop of peptone water in such a way that the center of the depression

lies over the drop. The slide was quickly inverted and viewed under the microscope, using oil immersion objective [11].

Catalase Test

Catalase test was used to check microorganisms that produce the catalase enzyme. A clear, grease-free slide was treated with 3% hydrogen peroxide (H_2O_2), and a small amount of each bacterial isolate was placed on the glass slide using a sterile inoculating loop, allowing for the isolate's bubbles to develop. Bubbles show catalase positive, while the absence means catalase negative [13].

Coagulase Test:

Coagulase is an enzyme that is capable of coagulating certain blood plasma notably human and rabbit plasma**.** This test differentiates pathogenic from non-pathogenic *Staphylococcus* spp., the test was carried out using 18–24-hours old culture. A loopful of isolated bacterium was emulsified with normal saline solution on a microscope slide. A drop of undiluted plasma was added to the suspension and stirred for five seconds. A coagulase-positive result was indicated by clumping of colonies together [11].

Oxidase Test:

Oxidase test is helpful in the identification of microorganisms that can produce cytochrome oxidase enzyme. The oxidase reagent was freshly prepared into a 1% solution, and filter paper strips were immersed. The culture was scratched with the inoculating wire loop. Positive reactions are indicated by a vivid, deep-purple hue that appears within 5–10 seconds, while adverse reactions are indicated by a lack of colour change [9].

Indole Test

The indole test is helpful in the identification of bacteria that can produce enzymes that convert tryptophane amino acid into indole gas. Tryptone broth (5 ml) was placed into different test tubes after which a loopful of the bacterial isolates was inoculated into the test tubes, leaving one of the test tubes uninoculated to serve as control. The test tubes were incubated at 37 ^{0}C for 48 hours. Then, 0.5 ml of Kovac reagent was added, shaken gently and allowed for 20 minutes to rise. Red colour at the top surface of the tube indicates a positive result while yellow colouration indicates a negative result [13].

Sugar Fermentation Test:

The isolates were tested for their ability to ferment sugars, primarily Gram-negative bacteria, to produce both acid and gas, or only acid, for differentiation purposes. Peptone water was prepared in a conical flask and phenol red indicator added. A Durham tube was provided in tubed broth media to collect the gas produced during fermentation. The tubes with their content were sterilized by autoclaving at 121ºC for 15 min. 1% solution of the sugar was prepared and sterilized separately at 115°C for 10 min. This was then aseptically dispensed in 5ml aliquot volume into the tubes containing the peptone water and indicator. The tubes were inoculated with young culture of the isolates and incubated at 37°C. Acid and gas production was observed after about 24hours of incubation. Acid production was indicated by the change of the medium from pale red to yellow colour, while gas production was indicated by the presence of gas in the Durham's tubes [13].

Citrate Test

This test detects the ability of an organism to use citrate as a sole source of carbon and energy. Citrate agar (2.4 g) was dissolved in 100 ml of distilled water. Ten milliliter (10 ml) of citrate medium will be dispensed into each tube and covered, then sterilized and allowed to cool in a slanted position. The tubes will be inoculated by streaking the organisms once across the surface. A change from green to blue indicates utilization of the citrate [9].

Methyl Red Test.

Some bacteria use glucose and convert it into other types of acids such as lactic acid, acetic acid and formic acid as last products. First of all, they covert glucose to pyruvic acid and then to different type of acids, this depends on the species of bacteria for which type of acid will be produced. Acid production decreases the medium pH that changes the color of the methyl red from yellow to red indicate the potential of bacteria to use glucose present in the culture medium. In this test five millimeters of glucose phosphate broth (1 g glucose, 0.5% KH2PO4, 0.5% peptone and 100 mL distilled water) was dispensed in a clean test tube and sterilized. The tubes were inoculated with the test organisms and incubated at 37^0C for 48 hours. At the end of incubation, few drops of methyl red solution were added to each test and colour change was observed. A red colour indicates a positive reaction [14].

Fungal Staining

The lactophenol cotton blue (LPCB) stain is the most widely used staining solution in the examination of yeasts and moulds. This serves as both a mounting fluid in wet mounts and a stain. It is simple to prepare. The preparation has three components: phenol, which will kill any live

organisms; lactic acid which preserves fungal structures, and cotton blue which stains the chitin in the fungal cell walls. Upon the addition of lactophenol cotton blue, fungi stain blue allows for easier visualization and examination (Abida, 2010). Two drops of lactophenol cotton blue were placed on clean glass slide and small piece of mycelium free of medium will be removed with sterile inoculating wire loop and transferred on to the stain. The mycelium was teased (picked) out with the needles and covered with clean cover slip carefully avoiding air bubbles and it was observed under the microscope for vegetative and reproductive structures [14].

Frequency of Occurrence Percentage Determination

Determination of microbial occurrence percentage was done to determine the frequency of occurrence of the different microbial isolates. Isolations were from the different fermented cayenne pepper sources and were cultured differently. The number of occurrences for each of the isolates in the four different markets was recorded, calculated and expressed as a percentage [15] .

Percentage frequency of occurrence = X/N x 100/1

X = Number of each organism in a cayenne pepper fruit from a particular market

N = Total number of the entire organism in the cayenne pepper fruits from a particular market

Mean = Average of an isolate from the four markets

Total = Summation of the whole isolate from a particular market.

Statistical Analysis

The data generated was subjected to one-way analysis of variance (ANOVA) to determine the differences in means of the bacterial isolates within the markets. All statistics were evaluated at $p< 0.05$ significance level.

RESULT AND DISCUSSION

The analysis of fermented cayenne pepper from the four markets within Enugu metropolis revealed a diverse microbial population with variations in their frequency of occurrence across different locations as seen below. The morphological and biochemical characteristic of bacteria isolated from fermented cayenne pepper samples are presented (Table 1). *Streptococcus lactis*, *Bacillus subtilis, Lactobacillus plantarum* and *Enterococcus faecalis* were the probable organisms isolated. Similar organisms were obtained in the past for spontaneous fermentation of *Capsicus* spp [7].

Furthermore, fungi were isolated and identified from the fermented cayenne peppers. The probable fungi were *Mucor* spp, *Aspergillus niger*, *Rhodotorulla* spp and *Geotricum* spp. (Table 2).

Table 1: Morphological and biochemical features of bacterial isolates of fermented cayenne pepper obtained from four different markets in Enugu metropolis.

Biochemical Feature	**BAC 1**	**BAC 2**	**BAC 3**	**BAC 4**
Shape	Cocci	Rod	Rod	Diplococci
Colony Setting	Clusters	Circular	Paired	Clusters
Colour	Creamy	Off-white	Whitish	Cream
Elevation	Elevated	Irregular	convex	Convex
Appearance	Opaque	Opaque	Shinny	Smooth
Catalase	-	-	-	-
Oxidase	-	-	+	+
Coagulase	-	-	-	-
Indole	-	-	-	-
Citrate	-	-	-	-
Oxidase	-	-	+	+
Motility	-	-	-	-
Gram staining	+	+	+	+
Probable Organism	*Streptococcus lactis*	*Bacillus subtilis*	*Lactobacillus plantarum*	*Enterococcus feacalis*

+ Positive, - Negative

Table 2 Cultural and Morphological characteristics of fungi and their tentative identification

isolated from fermented cayenne pepper sample obtained from four different markets within Enugu metropolis.

+Positive, -Negative

Cultural characteristics	**Morphological characteristics**	**Identification**
Large white colonies which turn into black later.	Erect sporangiophores are formed. Sporangiophores swells at the tip to form sporangia which are globular shaped.	*Mucor*
It has a dark-brown mycelium	Dark-brown conidia, conidiophores are long, globose vesicles that are completely covered with biseriate phialides, phialides are borne on brown metulae	*Aspergillus niger*
Red-pink colonies	Spherical to enlongated budding yeast-like cells or blastoconidia	*Rhodotorulla*
White creamy colonies	No conidia, branching hyphae, rectangular arthroconidia	*Geotricum*

Results for the total viable count of bacteria and fungi obtained from fermented cayenne pepper comparing samples from Ogbette, Abakpa, Orie Emene and Obiagu markets within Enugu metropolis are presented in Table 3. All samples showed active loads of bacteria and fungi. Ogbete

market had the highest bacteria count of 6.67 x 10^3 CFU/ml followed by Orie Emene (4.21 x 10^3 CFU/ml), while Abakpa market exhibited the least bacterial count of (1.68 x 10^3 CFU/ml). A similar trend was observed for fungi count ranging from 5.53 x 10^3 to 2.16x 10^3 CFU/ml for Ogbete and Abakpa markets respectively. Total microbial count of 12.20 x 10^3, 7.84 x 10^3, 4.74 x 10^3 and 3.34 x 10^3 1.68 x 10^3 CFU/ml were observed from fermented cayenne pepper obtained from Ogbete, Orie Emene, Obiagu and Abakpa market, respectively. Furthermore, bacteria count equals 52.01 % of the total viable count while fungi count was 47.99 %. This viable microbial count is evident of the hygienic condition of the markets as well as the storability of the cayenne pepper before sale. This indicates how many microbes had been acquired internally by the samples, such that effective washing before blending and fermentation still allowed for a highly viable microbial count after fermentation [10,16]. This trend could be explained by increasing microbial load with time [1].

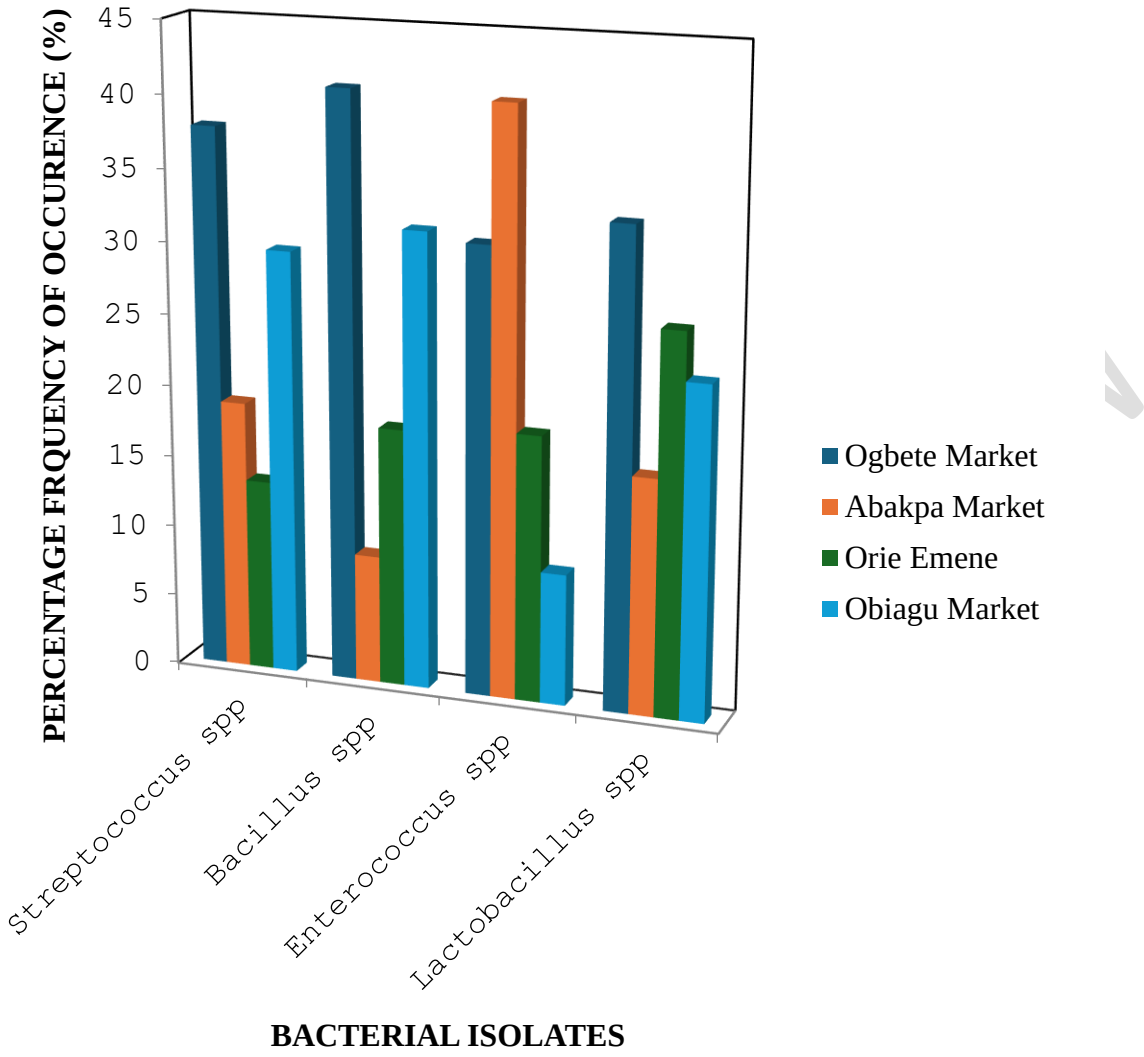

Figure 1: Percentage frequency of occurrence for bacteria in fermented cayenne pepper from the four different markets in Enugu metropolis

The percentage frequency of bacteria occurrence isolated from fermented cayenne pepper samples gotten from four different markets in Enugu metropolis (Figure 1). *Streptococcus* spp, *Bacillus* spp, *Enterococcus* spp and *Lactobacillus* spp. were isolated from all the samples. For *Streptococcus* spp., highest occurrence of 37.83 % was observed in Ogbete market, followed by Obiagu market (29.73 %) and least occurred in Orie Emene market (13.51 %). *Bacillus* spp.,

exhibited a similar trend as *Streptococcus* spp., ranging from 40.91 to 9.09 %. However, *Enterococcus* spp. differed by exhibiting the highest occurrence in samples obtained from Abakpa market with 40.63 %, next was Ogbete market (31.25 %) and least was Obiagu market with 9.38 %. Lastly, *Lactobacillus* spp. ranged from 33. 33 to 16.67% for Ogbete market and Obiagu market, respectively The presence of these bacteria is consistent with other studies on fermented products, where lactic acid bacteria such as *Lactobacillus* and *Enterococcus* are often dominant due to their role in fermentation and acid production [17]. Also, Ogbete showed the highest occurrence of most organisms because of its active nature and size, resulting from intensive footfall. Identifying these bacteria aligns with their known roles in the fermentation process, where they contribute to flavor development, acid production, and preservation of fermented foods [18].

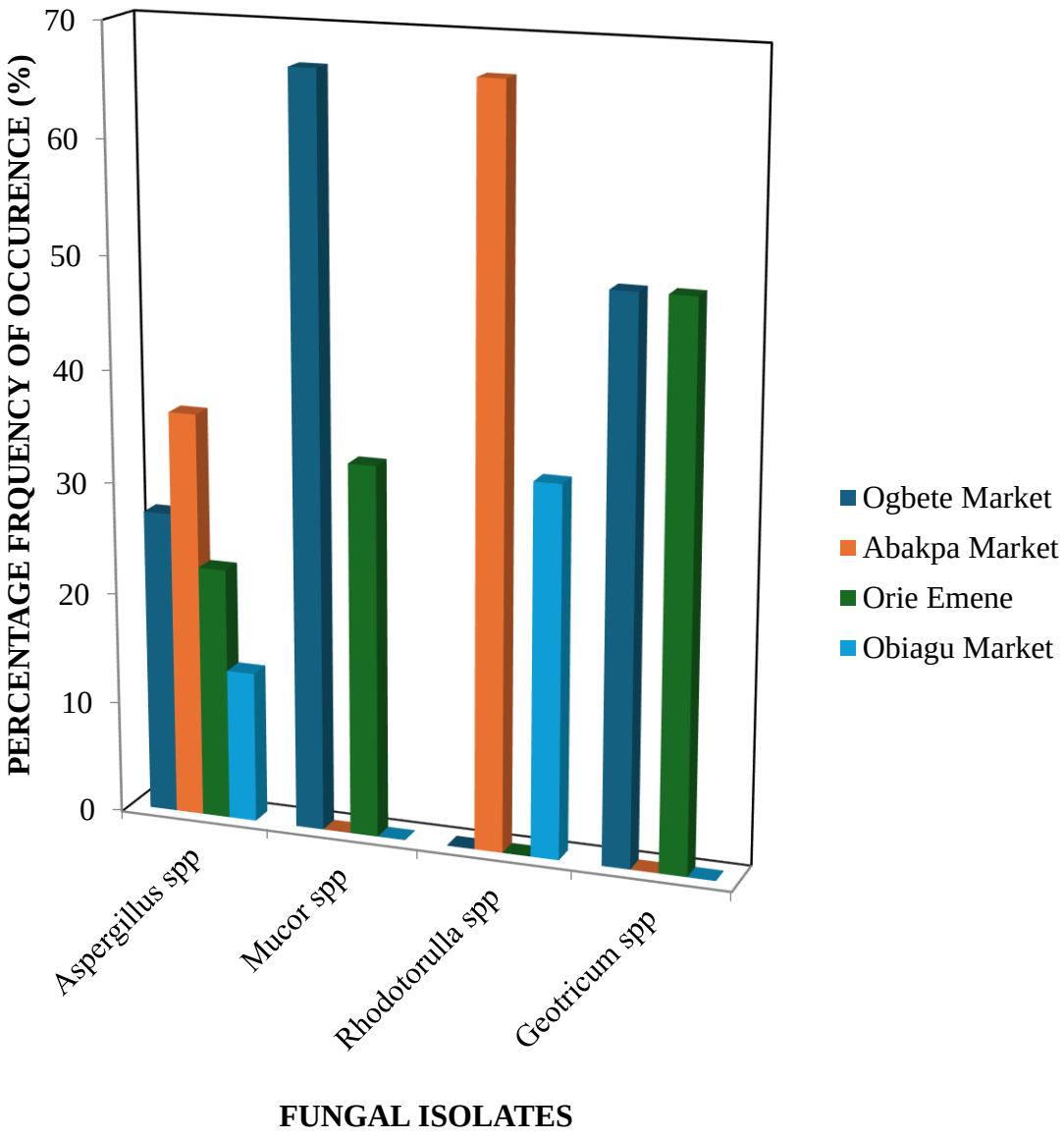

Figure 2: Percentage frequency of occurrence for fungi in fermented cayenne pepper from the four different markets in Enugu metropolis.

The percentage occurrence of fungi isolated from fermented cayenne pepper sold in four different markets at Enugu metropolis (Figure 2). The isolated fungi were *Aspergillus* spp, *Mucor* spp,

Rhodotorulla spp and *Geotricum* spp. Samples obtained from all the markets exhibited the presence of *Aspergillus* spp of which Abpkapa market had the highest occurrence of 36.36%, followed by Ogbete market with 27.27%, next was Orie Emene market (22.73%) while Obiagu market showed least occurrence of 13.64%. Abakpa and Obiagu shown no occurrence of *Mucor* spp, while its frequency of occurrence in Ogbete (66.67%) doubled that of Orie Emene (33.33%). A similar trend was observed for *Geotricum* spp, however, an equal occurrence of 50% each was counted for Ogbete and Orie Emene market. On the contrary, an opposite trend was observed for *Rhodotorulla* spp, with no occurrence in Ogbete and Orei Emene markets but showed highest occurrence in Abakpa market (66.67%) and least occurrence in Obiagu market (33.33%). The presence of these fungi spp in the samples, indicates the possibility of certain hygienic conditions in the markets. For instance, the presence of *Aspergillus* spp. is of concern because it can produce aflatoxins, which are harmful mycotoxins [19]. The varied distribution of these fungi could be due to differences in environmental conditions, handling practices, and fermentation processes in each market.

Table 3**:** Microbial frequency of occurrence of bacteria and fungi isolates from fermented cayenne pepper samples from Abakpa, Obiagu, Ogbete and Orie Emene markets.

Bacteria	Ogbete Market %	Abakpa Market %	Orie Emene market %	Obiagu market %	Mean
Streptococcus spp	32.08	21.88	17.24	35.48	26.67
Bacillus spp	16.98	6.25	13.79	22.58	14.90
Enterococcus spp	18.87	40.63	20.69	9.68	22.47
Lactobacillus spp	18.87	15.63	27.59	22.58	21.16
Aspergillus spp	5.66	9.38	10.34	6.45	7.96
Mucor spp	3.77	-	3.45	-	1.81
Rhodotorulla spp	-	6.25	-	3.23	2.37
Geotricum spp	3.77	-	6.90	-	2.67
Total	100	100	100	100	100

An overview of the microbial frequency occurrence of fermented cayenne pepper sold in four different markets at Enugu metropolis (Table 3). Exhibiting the percentage sharing between bacteria and fungi and mean frequency occurrence of each organism across all the market. From

the observed score bacteria and fungi spp. showed comparable occurrence in all the markets. For instance, total bacteria and fungi spp. scores are 86.79 and 13.22 %, 84.38 and 15.63 %, 79.31 and 20.69 %, lastly 90.32 and 9.68% for Ogbete, Abakpa, Orie Emene and Obiagu markets, respectively. This indicates that the bacterial occurrence was significantly higher across all the market with frequency values above 79% in all the cases. While fungi occurrences were below 21% in all the markets. The mean frequency occurrence for fermented cayenne pepper sold in four different markets at Enugu metropolis of each organism presents *Streptococcus* spp as the highest occurring species (26.67%), followed by *Lactobacillus* spp (21.16 %) while *Mucor* spp exhibited the least occurrence of 1.81%.

The variations in microbial populations across different markets reveal the influence of local environmental conditions and fermentation practices on the microbial ecology of fermented foods [4,20]. This study adds to the growing body of knowledge on the microbial diversity of traditional fermented products, providing valuable insights for improving fermentation processes and ensuring food safety [21].

CONCLUSION

This study on fermented cayenne pepper samples in Enugu metropolis revealed a diverse range of bacteria and fungi, highlighting the complexity of microbial communities. The Ogbette market had the highest bacterial and fungal loads, indicating local environmental conditions and fermentation processes. The presence of beneficial lactic acid bacteria and fungi emphasizes the need for food safety measures. Future research should focus on optimizing fermentation processes and exploring microbial functional properties to enhance nutritional and health benefits.

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