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Microbiological Assessment of Sun-Dried Beef (*Tinko*) Sold in Some Markets in Port-Harcourt Metropolis, Rivers State

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ABSTRACT

The safety of sun-dried beef (*Tinko*) is critical for public health since it is a popular and commonly eaten food. This study investigated the bacterial and fungal contamination of dried beef samples towards evaluating the overall safety of this food product. A total of eighteen (18) dried beef samples were purchased from six major markets in Port Harcourt, Nigeria and transported immediately to NSPRI, Port-Harcourt for microbiological analysis. Identification of bacteria and fungi isolated from the samples was determined based on standard methods. Statistical analyses were performed using SPSS 20.0. Results showed that there was significant difference $p < 0.05$ in both bacteria and fungi counts with higher bacterial counts at 10^5cfu/g compared to fungi counts at 10^3cfu/g recorded. The bacterial counts of dried beef from Oil Mill ($5.10 \pm 0.30 \times 10^5$cfu/g) and Rumuokoro ($7.85 \pm 0.65 \times 10^5$cfu/g) markets were above the International Commission of Microbiological Specification for Food (ICMSF) maximum microbiological limit of 5×10^5cfu/g. Identified bacterial isolates were *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus*, *Streptococcus* sp and *Pseudomonas aeruginosa*. *Bacillus subtilis* (22.32%) and *E. coli* (7.29%) were the highest and least occurring bacteria respectively with the highest frequency recorded in Rumuokoro market (35.75%) and the least from Creek Road market (5.47%). Similarly, identified fungal isolates were *Mucor mucedo*, *Aspergillus niger*, *Penicillium notatum*, *Rhizopus stolonifer* and *Cladiosporium* sp. *Rhizopus stolonifer* (30.51%) and *Penicillium notatum* (10.17%) were the highest and least occurring fungi respectively with the highest frequency recorded in Rumuokoro market (38.99%) and the least from in both Creek Road market and Mile 1 market (8.47%). The presence of these potential pathogens in the beef samples emphasizes the significance of routine quality control and microbiological monitoring in the Port-Harcourt dried beef supply chain as to protect food safety and public health.

Keywords: Sun-dried beef, markets, microbiological monitoring, bacteria, fungi, food safety, public health

Introduction

Animal products, such as meat, contribute to both human and animal diets owing to their excellent nutritional properties, delivering high quality protein, vitamins, and minerals (Ike and Akortha, 2017). However, due to its high nutritional value, it is perishable and provides the ideal conditions for the growth of spoilage microorganisms, particularly bacteria and moulds.

In Nigeria, sun-dried meat (*Tinko*) from various domestic and wild animals are frequently consumed and is sold by many vendors at markets, motor parks, along busy roads and strategic areas all over the nation. However, the quality and safety of these dried meats is an issue that requires regular investigation and study by governmental, non-governmental and academic bodies due to the potential presence of pathogenic microorganisms that can cause foodborne illnesses (Ada *et al*., 2022). Thomas (2012), documented that meats contains high amounts of proteins which putrefactive bacteria can utilize for growth and carbohydrate which the bacteria can further utilize for energy. Additionally, meat contains high water content that is essential for bacteria growth. As earlier documented by Prescott *et al*. (2002), the growth of microorganisms is impossible in food products with

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water activity values lower than 0.7. Several studies have reported the application of drying methods for the preservative and shelf-life extension of meat. According to Kehinde *et al*. (2021), meat quality assurance is increased in accordance with the measure of the microbial load assessment.

Adeyeye (2016) claims that in order to ensure that beef products have a longer shelf life; new processing techniques have had to be developed. Meat is preserved by refrigeration, freezing, and heat processing on a global scale; however certain traditionally processed meat products are still in demand, particularly in underdeveloped nations with little access to electricity. Kehinde *et al*. (2021) also pointed out that various food processing methods, such as boiling, frying, drying, and grilling, are used to preserve fresh meat and add value to the supply chain, particularly in underdeveloped and emerging cultures.

In many regions of the world, street sellers provide dried beef, a widely consumed meal. However, the safety and quality of these products are often questionable due to the unhygienic conditions in which they are prepared and sold. Consumers may be seriously at risk for health problems if dried beef is contaminated with bacteria and fungi. To evaluate the microbiological quality of dried beef offered by vendors, several investigations have been done. The microbiological quality and dominating microorganisms of biltong produced in butcheries were also examined by Matsheka *et al*. (2014). The research discovered that the total viable count of biltong samples varied from 2 to 7 log10 cfu/g. The study also discovered that *Staphylococcus* and *Bacillus* were the most common bacteria associated with the biltong.

The sale of dried meat is a common site at strategic busy areas and open markets in Port-Harcourt. Products made from dried beef are typically offered for sale in open marketplaces, busy streets and at certain fast-food restaurants. The manners in which they are offered for sale to the general public, together with the handling procedures, raise concerns about the items' level of hygienic condition (Ajiboye *et al*., 2011). The items are put on exposed tables or trays that are readily contaminated by hands from potential customers, insects, sand, exhaust smoke, and other contaminants. According to Bryan *et al*. (1992), food-borne disorders are illnesses brought on by ingesting bacteria, toxins, or cells generated by microorganisms found in food. Most consumers who rely on dried meat are more concerned with its convenience than its safety, quality, or cleanliness (Duff *et al*., 2003; Draper, 1996). In these situations, microbiological analysis is frequently extremely helpful in determining the safety of dried meat products and preventing public health risks among consumers. Microbiological safety is the biggest issue with street or open market dried meat, particularly because the preparation is done in locations that could have inadequate sanitation. According to Shehu *et al*. (2020), the types and quantities of microorganisms that are present in a completed food product are generally impacted by the bacteriological environment in which the food was first produced; Quality of the food in its raw or unprocessed state, the sanitary conditions under which the product is handled and processed and the adequacy of subsequent packaging, handling, and storage conditions of the products (Tinker, 1985). These are factors that contribute to the microbiological quality of the dried meat product.

The aim of this study is to assess the bacteria and fungi present in sun-dried beef samples sold in major markets in Port-Harcourt, metropolis in Rivers State, Nigeria; towards evaluating the overall safety of this food product and its implication for public health.

Materials and Methods

Locations (markets) and Collection of Samples

Six (6) different major markets were considered for this study are denoted as Market A (Creek Road), Market B (Choba), Market C (Rumuokoro), Market D (Mile 1), Market E (Rumuokuta) and Market F (Oil Mill), all within Port-Harcourt Metropolis, Rivers State.

Three (3) dried beef samples each weighing approximately 30g, were randomly purchased from six (6) markets and different selling shades according to Shehu *et al*. (2020) and placed in small sterile polyethylene packs according to Whitaker, (2003). A total of eighteen (18) samples of dried meat were obtained from the markets. The dried beef samples were transported immediately to the Nigerian Stored Products Research Institute (NSPRI), Port-Harcourt Microbiology Laboratory for analysis using standard microbiological techniques.

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Cultivation, Enumeration and Isolation of Bacteria

Total heterotrophic bacteria were determined by inoculating the surface of sterile dried Nutrient Agar (NA) plates with aliquot (0.1 ml) of 10^{-4} dilutions in duplicates. The aliquot was evenly spread using sterile bent glass rod followed by incubation of the inoculated plates in the incubator at 37°C for 24 h. After incubation, colonies that grew on the respective plates were enumerated and used in assessing the bacterial counts of the various beef samples. Characterization of bacterial isolates was determined based on their cultural and morphological characteristics. Morphological characteristics adopted include color, shape, texture, size of colonies, and gram staining technique while the biochemical characteristics were Methyl Red (MR) test, Voges Proskauer (VP), catalase, oxidase, citrate tests and sugar fermentation tests. Identities of bacterial isolates were further authenticated by referencing the observed and recorded characteristics with those documented in the Bergy's manual of determinative biology (Prescott *et al*., 2011).

Enumeration and isolation of total heterotrophic fungi

Sabouraud Dextrose Agar (SDA) (Titan Biotech Ltd., India) was used to determine total heterotrophic fungi count. In this study, spread plate method was applied as described by Prescott *et al*. (2011). An aliquot (0.1 ml) from 10^{-2} of the serially diluted samples was inoculated in duplicates onto surface of dried SDA plates and then spread evenly with a flamed glass spreader. The plates were incubated at 25 °C for 72 h after which the colonies were enumerated and recorded (Iyeritei and Obire, 2022). A portion of the fungal growth were picked using flamed inoculating pin and placed on a clean glass slide containing a drop of Lactophenol Cotton Blue (LPCB) and gently teased with the inoculating pin. The preparation was covered with a cover slip then observed with the aid of an electron microscope (OPTIKA, Italy). The presence or absence of septa in the mycelium, type of spore, presence of primary or secondary sterigmata, and other microscopic characteristics as well as cultural characteristics were used in the identification of the fungal isolates. These isolates were ascertained by comparison with a fungal atlas (Kidd *et al*., 2016).

Data Analysis

Statistical analyses were performed on the data obtained using SPSS 20.0. One-way Analysis of Variance (ANOVA) test was used for the comparison of means of Total Viable Count (bacterial load and fungi load) across sampling while percentage frequency of occurrence was calculated. The means were separated for test of significance by the Duncan's Multiple Range Test at $P < 0.05$.

Results

The result of the total viable microbial count of dried beef from various market vendors in Port-Harcourt metropolis is shown in Table 1. There was significant difference $p < 0.05$ in both bacteria and fungi count with higher bacterial counts at 10^5cfu/g compared to fungi counts at 10^3cfu/g. The highest bacterial count ($7.85 \pm 0.65 \times 10^5$cfu/g) and highest fungi count ($2.30 \pm 0.20 \times 10^3$cfu/g) were recorded in Market C (Rumuokoro Market). Similarly, the least bacterial count ($1.20 \pm 0.10 \times 10^5$cfu/g) and least fungi count ($0.50 \pm 0.20 \times 10^3$cfu/g) were recorded in Market A (Creek Road Market).

Table 1: Total viable microbial counts of dried beef from various markets in Port-Harcourt

Market	*Total Viable Microbial Count	
	Bacterial Count (x 10^5cfu/g)	**Fungi Count (x 10^3cfu/g)**
Creek Road (A)	1.20 ± 0.10^{a}	0.50 ± 0.20^{a}
Choba (B)	2.95 ± 0.55^{bc}	0.95 ± 0.05^{a}
Rumuokoro (C)	7.85 ± 0.65^{e}	2.30 ± 0.20^{b}
Mile 1 (D)	1.65 ± 0.15^{ab}	0.50 ± 0.10^{a}
Rumuokuta (E)	3.20 ± 0.50^{c}	1.00 ± 0.10^{a}
Oil Mill (F)	5.10 ± 0.30^{d}	0.65 ± 0.25^{a}

Legend: Values are mean and standard error of triplicate counts, a-d: different characters in the same row indicate values with significant difference ($p>0.05$)

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Figure 1 shows result of the percentage frequency of occurrence of bacteria isolates from the dried meat samples. The bacterial isolates were *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus*, *Streptococcus* sp and *Pseudomonas aeruginosa*. Percentage frequency of occurrence as shown in the figure shows that the highest occurring bacteria was *B. subtilis* (22.32 %) followed by *B. cereus* (19.37 %) while the least occurring was *E. coli* (7.29%). Percentage occurrence of other bacteria was *Pseudomonas aeruginosa* (15.26%), *Streptococcus* sp (14.12%), *M. luteus* (11.39%) and *S. aureus* (10.25%).

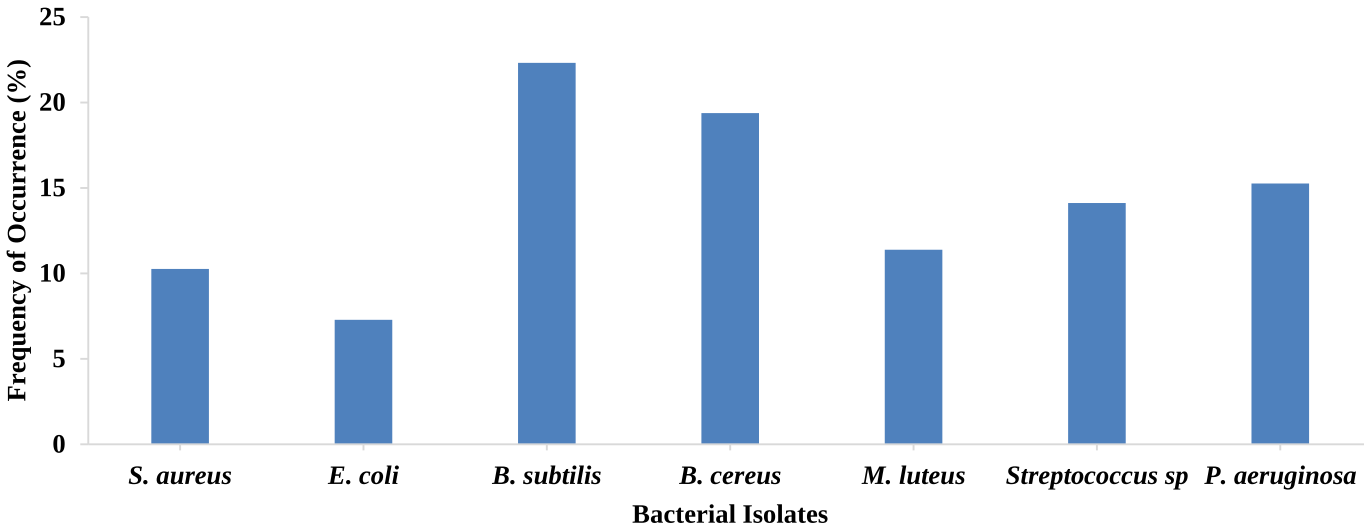

Fig. 1: Total Frequency of occurrence (%) of bacterial isolates from the dried meat samples

Figure 2 shows the percentage frequency of occurrence of fungi isolates from the dried meat. As shown in the figure, five (5) fungi were isolated from the dried beef. The fungi isolates were *Mucor mucedo*, *Aspergillus niger*, *Penicillium notatum*, *Rhizopus stolonifer* and *Cladiosporium* sp. Percentage frequency of occurrence as shown in the figure shows that the highest occurring fungi was *R. stolonifer* (30.51 %) followed by *M. mucedo* (24.58 %) while the least occurring was *P. notatum* (10.17%). Percentage occurrence of other fungi was *Cladiosporium* sp (23.73%) and *A. niger* (11.01%).

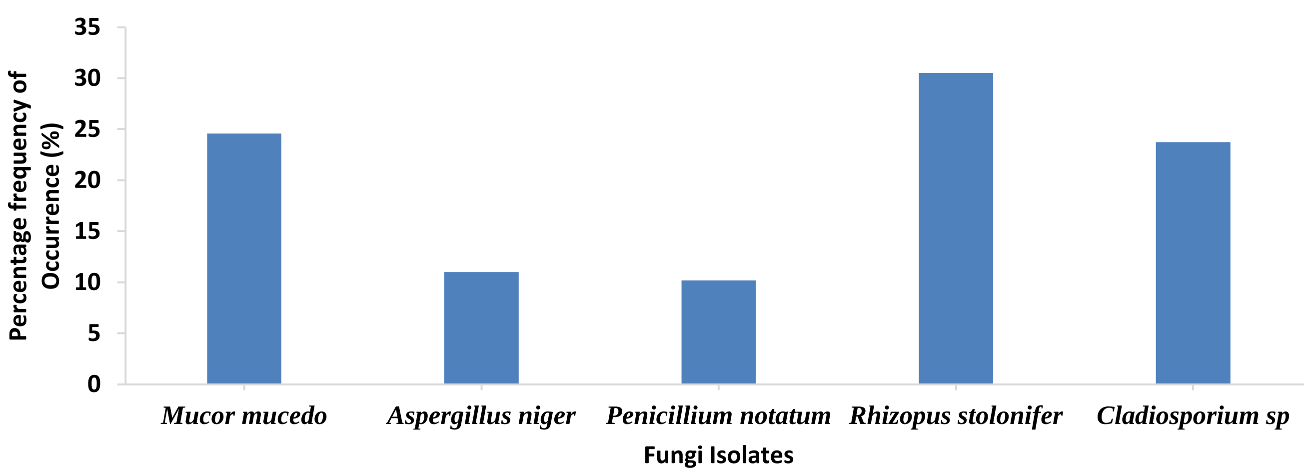

Figure 2: Total frequency of occurrence (%) of bacteria isolates from the dried meat samples

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Table 2 shows percentage frequency of occurrence of bacteria and fungi colonies in the dried beef obtained from the various markets. As shown in the table, the highest percentage frequency of occurrence of bacteria was recorded in Market C (Rumuokoro market = 35.75 %) followed by Market F (Oil Mill market = 23.23 %) while the least percentage occurrence of bacteria was recorded in dried beef samples from Market A (Creek Road market = 5.47 %). Other values were Market E (Rumuokuta market = 14.58 %); Market B (Choba market = 13.44 %) and Market D (Mile 1 market = 7.52 %). Table 2 also shows the frequency of occurrence of fungi isolates obtained from the various markets. The highest percentage frequency of occurrence of fungi in the dried beef samples was recorded in Market C (Rumuokoro market = 38.99 %) followed by Market E (Rumuokuta market = 16.95 %) while the least percentage occurrence of bacteria was recorded in dried beef samples from Market A and D (Creek Road and Mile 1 markets = 8.47 %). Other values were Market B (Choba market = 16.10 %) and Market F (Oil Mill market = 11.02 %).

Table 2: Frequency of occurrence (%) of bacteria and fungi colonies in the dried meat from the markets

Market	**Frequency of occurrence**			
	Bacteria		**Fungi**	
	Occurrence (N = 439)	**Percentage (%)**	**Occurrence (N = 118)**	**Percentage (%)**
Creek Road (A)	24	5.47	10	8.47
Choba (B)	59	13.44	19	16.10
Rumuokoro (C)	157	35.76	46	38.99
Mile 1 (D)	33	7.52	10	8.47
Rumuokuta (E)	64	14.58	20	16.95
Oil Mill (F)	102	23.23	13	11.02

Discussion

This present study has revealed the population and types of bacteriaand fungi present in sun-dried beef samples sold in major markets in Port-Harcourt, metropolis in Rivers State, Nigeria. In this present study, higher bacterial count was recorded in dried beef purchased from Choba (2.95±0.55 x 10^5cfu/g), Rumuokuta (3.20±0.50 x 10^5cfu/g), Oil Mill (5.10±0.30 x 10^5cfu/g) and Rumuokoro (7.85±0.65 x 10^5cfu/g) markets. Similarly, higher fungi count was recorded in dried beef purchased from Oil Mill (0.65±0.25 x 10^3cfu/g), Choba (0.95±0.05 x 10^3cfu/g), Rumuokuta (1.00±0.10 x 10^3cfu/g) and Rumuokoro (2.30±0.25 x 10^3cfu/g) markets. According to the International Commission of Microbiological Specification for Food (ICMSF, 1986), the maximum microbiological limit (total viable count) which separates the good quality meat from the bad quality is 5 × 10^5cfu/g. In this study, the bacteria and fungi counts were above the acceptable limit in dried meat samples purchased from Oil Mill and Rumuokoro markets. In addition, significantly different and higher bacteria and fungi contamination in the various purchased beef samples could be associated to the proximity of the markets to major commercial motor parks in Port-Harcourt. Garba *et al*. (2021), reported that the high microbial load in dried fish sample purchased from various markets could be associated to the location of the market which was observed to be highly polluted due to anthropogenic activities, such as lack of proper drainage system, refuse dump, improper hygienic and handling procedures adopted by the sellers at the various markets. In comparison with other studies, Ada *et al*. (2022) reported lower aerobic bacteria count range of 3.40 × 10^4 cfu/g to 9.10 × 10^4 cfu/g in the three points studied. In another study by Jabaka *et al*. (2021), much lower bacterial load ranging from 8.00 × 10^2 cfu/g to 3.50 × 10^3 cfu/g was reported and was attributed to the sample being freshly prepared. In addition, lower bacterial and fungal load was reported by Olayemi *et al*. (2012) when testing the effectiveness of NSPRI developed smoking kiln which was documented to effectively reduce microbial spoilage of smoked fish. On the other hand, Plavsic *et al*. (2015) reported higher moulds at 1.20 x 10^6 cfu/g and yeast at 1.00 x 10^7 cfu/g in the dried smoked meat.

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This higher bacterial count in the dried meat has been reported to occur immediately in the absence of refrigeration and that refrigeration slows or stops microbial growth.

The presence of such high bacterial and fungal counts as recorded in this present study suggests potential food safety risks which can lead to food spoilage and, more importantly, can pose health hazards to consumers if pathogenic microorganisms are present. Furthermore, the variations in microbial counts among different markets may be attributed to differences in handling, storage conditions, and hygiene practices in these locations. Several factors have been reported to contribute to high microbial counts in dried foods, such as inadequate unhygienic handling, and prolonged storage periods indicating the need for improved food safety practices and stricter regulations in this market (Ada *et al*., 2022). The significant differences in microbial counts among the markets highlight the importance of monitoring and regulating the food supply chain. Market-specific factors, such as vendor practices, storage facilities, and transportation, likely contribute to these variations. Conclusively, the significant differences in bacterial and fungal counts among dried beef samples from different markets in Port-Harcourt emphasize the need for improved food safety measures, especially in markets with higher microbial loads.

Bacteria isolated in this present study with the highest occurring were *B. subtilis* followed by *B. cereus* while the least occurring was *Escherichia coli*. Other bacterial isolates were *Pseudomonas aeruginosa*, *Streptococcus* sp, *Micrococcus luteus* and *Staphylococcus aureus*. The high occurrence of *Bacillus subtilis* and *Bacillus cereus* suggests that these spore-forming bacteria may have survived the drying process and could pose a risk if the beef is not properly stored or prepared. Escherichia coli is a type of bacteria that can cause diarrhea, urinary tract infections, and other illnesses. The occurrence and isolation of E. coli which is a coliform, calls for health concern since it is associated with gastrointestinal infections (Collins *et al*., 2000). Occurrence of *Staphylococcus aureus* is also of concern as well, given their potential to cause foodborne illnesses (Mediani *et al*., 2012) as it is an indication of possible contamination from human sources. Staphylococcus aureus is a normal flora of the skin of man and can be transmitted from person to product through unhygienic practices.

Earlier study of Adesiyun *et al*. (1986), reported that enterotoxin producing strains of *S. aureus* have been isolated from food handlers and the possibility of their transmission to food was noted. *Micrococcus luteus, Streptococcus* sp, and *Pseudomonas aeruginosa* were also detected, indicating the diverse microbial population present in the dried beef samples. *Micrococcus luteus* is a common bacterium found in soil and water, and it is not typically associated with human illness while *Streptococcus* sp is a genus of bacteria that includes many different species, some of which can cause infections such as strep throat and pneumonia indicating its human source to the beef post-production (Raji, 2006). *Pseudomonas aeruginosa* is a common bacterium that can cause infections in people with weakened immune systems.

According to Raposo *et al*. (2016) and Kim *et al*. (2021), *Pseudomonas* spp., are spoilage bacterium with the innate ability to metabolizes glucose, amino acid and lactate resulting to the formation of slime and generation of off-odor. The isolation of *Pseudomonas* species from dried beef samples in this study indicates possible post-production contamination as these organisms are expected to have been destroyed during the high temperature treatment of cooking and drying. However, gram negative bacteria especially *Pseudomonas* has been reported as dominant meat spoilage organisms (Raji, 2006).

While these bacteria may not always be pathogenic, their presence warrants attention as they could influence the product's safety and shelf-life. The findings of this study have underscores the importance of proper hygiene, storage, and processing practices in the production and handling of dried beef to minimize the risk of contamination with pathogenic bacteria. Additionally, it highlights the need for regular monitoring and quality control measures in the meat processing industry. Furthermore, the varying percentages of bacterial occurrence suggest that specific control measures may need to be tailored to target the predominant bacteria in dried beef products. This information can aid in the development of targeted intervention strategies to improve the safety and shelf-life of dried meat products.

The fungi isolated from sun-dried beef samples in this study were *Mucor mucedo*, *Aspergillus niger*, *Penicillium notatum*, *Rhizopus stolonifer* and *Cladiosporium* sp.

The presence of these fungi in dried meat products can be attributed to improper storage conditions, such as high humidity and temperature, which promote fungal growth. Similar studies have been conducted on the occurrence of fungi in dried meat and fish products, and the results of this study are consistent with previous findings. Tafinta *et al*. (2023) reported isolating *A. flavus, A. niger, A. fumigatus* and *A. terreus* from dried meat sold at Sokoto Metropolis. In another study, Adeyeye (2016) reported isolating *A. flavus, A. niger, A. tamarri, Fusarium compacticum, F. oxysporum, F. sacchari, Penicillium chrysogenum, P. citrinin* and *P. oxalicum*. Ajiboye *et al*. (2011) reported isolating *Aspergillus niger, Aspergillus flavus, Penicillium* sp. and *Rhizopus* sp. from dried meat (*Tinko*) sold in Ilorin, Nigeria. Similarly, Nwaehujor *et al*. (2022) reported isolating *A. flavus, A. niger, Penicillium* sp., *Rhizopus* sp., Mucor sp. and yeast sp. from maize, groundnut and cowpea purchased from six major markets in Port Harcourt.

The presence of fungi in dried beef is a cause for concern, as some fungi can produce mycotoxins that can be harmful to human health. Tabuc *et al*. (2004), earlier reported that *Mucor mucedo* has been found to produce mycotoxins, which can cause respiratory and gastrointestinal problems while *Aspergillus niger* and *Penicillium notatum* were reported to be notable producers of ochratoxin A and patulin toxins, which have been linked to kidney damage and carcinogenic effects respectively. The implication of these findings is that the occurrence of toxin producing fungi in sun-dried beef which can be referred to as ready-to-eat dried beef, indicates the need for proper storage and handling practices to prevent the growth of these fungi and the production of mycotoxins. Consumers should also be aware of the potential health risks associated with consuming sun-dried beef that has been contaminated with fungi. Food safety regulations should be enforced to ensure that meat products are free from harmful fungi and mycotoxins. The presence of potential bacteria and fungi pathogens in the beef samples emphasizes the significance of routine quality control and microbiological monitoring in the Port-Harcourt dried beef supply chain as to protect food safety and public health.

In conclusion, the microbiological quality of dried beef purchased from several markets in Port-Harcourt metropolis, revealed that there is a considerable variation in both bacterial and fungal counts between the markets. This shows that dried beef in Port-Harcourt may be more susceptible to bacterial contamination for a variety of reasons, including handling procedures, storage conditions, and hygiene requirements in diverse markets. *Bacillus subtilis* and *Bacillus cereus* were found to be the most commonly in the dried beef samples, while *Rhizopus stolonifer* was the predominant fungus species, according to the identification of particular bacteria and fungi isolates.

The results of this study suggest that in order to reduce the spread of these possibly hazardous microorganisms during the processing and storage of dried beef, cleanliness and food safety procedures should be enhanced. Furthermore, it is interesting to examine the differences in microbial prevalence between the marketplaces. It was clear that both for bacteria and fungus, Rumuokoro Market regularly had the greatest microbial numbers. This finding shows that food safety enforcement and regulation in this industry must be given more consideration. This study emphasizes the significance of routine quality control and microbiological monitoring in the Port-Harcourt dried beef supply chain. Consuming large amounts of dried beef has certain health concerns, therefore it's important to make an effort to improve food safety procedures and teach market sellers and consumers about correct handling and storage methods. Future research might focus further on the sources of pollution and any possible health effects to offer a more thorough knowledge of this problem.

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Author contributions

Isiekwene, A.C. and Salami, D.O. designed the work and also did the literature search; Ikeorah, N.N. and Salami, D.O. sourced, purchased and checked the samples for entomological parameters before purchasing; Isiekwene, A.C., Inana, M.E., Ekeocha, E.C., Eke, S.I. and Israel, D.U. carried out the microbial analysis; Inana, M.E. and Awagu, E.F. supervised the work; Isiekwene, A.C. also wrote the manuscript. All authors read and approved the final manuscript.

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