ASSESSMENT OF MICROBIOLOGICAL QUALITY ASSOCIATED WITH READY-TO- EAT BUSH MEAT SOLD AT RUMUOKORO MARKET IN RIVERS STATE

ABSTRACT

The study was carried out to investigate the Microbiological quality of microorganisms associated with ready to eat bush meat sold at Rumuokoro market in Rivers state. Total numbers of 24 samples were collected and analysed using different media such as Nutrient agar for Total aerobic plate count (TAPC), MacConkey agar for coliform count, Eosin methylene blue for Escherichia coli (EC) and Potato Dextrose Agar for Fungal count (FC) and folds serial dilution was used. Four different microorganisms were isolated such as *Staphylococcus* spp, *Pseudomonas* spp, *Bacillus* spp and *Escherichia coli.* The total aerobic plate count (TAPC). *E. coli* count (EC). Coliform count (CC) and Fungal count (FC) isolated from antelope were higher when compared to grass cutter so there was significant difference (P <0.005). The occurrence of *Staphylococcus aureus* isolated from antelope (26.9%) was higher when compared to grass cutter (25.0%). However the occurrence of *Pseudomonas* spp and *Bacillus* spp isolated from Antelope (23.1% and 30.8%) were higher when compared to grass cutter (12.5% and 18.5%) while the occurrence of the above organisms isolated on both Antelope is significantly difference (P<0.005) from grass cutter. But the occurrence of *Absidia* spp and *Penicillium* spp were higher in grass cutter sample (57.1%) and (42.9%) compared to antelope (55.6%) and (44.4%) respectively, although the mean difference was statistically significant (P<0.005) so there was significant difference. It is hereby recommended that most handlers should always wash hands before and after handling the meat as improper hand washing is the number one cause of food borne illness. Consumers of such meat should learn food hygiene practices such as, soaking the meat in warm salt solution, proper washing and well cooked before consumption.

Key words: Microbiological, Bush Meat, Food Hygiene, Grass cutter, Consumers.

1. Introduction

The forest and woodland is often refers to as bush, so the wild animals derived from the bush are being hunt and consumed as bush meat (James and Sam, 2007). Bush meat constitutes a vast array of species ranging from Donkeys, Leopard, Monkeys, Grass cutter (Thryonomys, Swindenanns), African elephant, Antelope (Alcalaphinae) (James and Sam, 2007).

Most Ready-to-eat bush meats sold in market especially in West Africa are usually those produced locally by drying with smoke and cooking. Hence, the method does not make the

meat totally free from microbial attack such as bacteria and fungi or toxic substances produced by theses bacteria, thus posing serious health threat or danger to the individual or group of individuals who basically rely on this type of meat as a source of food, particularly when not adequately cooked before consumption (James and Sam, 2007).

Similarly, Ready-to-eat bush meat usually serves as source of income as it can be exported or sold within to generate capital or money, cheaper protein source thereby resulting into massive consumption than alternative source of protein. Bush meat can be ready-to-eat when they are properly dried which is a complex process with many crucial steps starting from the slaughtering of the animal, carcass trimming selection of the raw material, proper cutting and pre- treatment of the pieces to be dried (Oliver *et al.,* 2009). More also, Ready- to-eat bush meat with a high fat content should not be kept for a long duration but utilized as soon as possible after cooking in order to avoid intensive rancidity (Fa *et al*., 2002).

Furthermore, these bush meat must be continuously examined for spoilage related off- odour, which is the result of incorrect preparation and or drying of the meat. Bush meat with signs of deterioration must be rigorously sorted out and not to be cooked (Oliver *et al.,* 2009).

In the same vein, there are some crucial noticeable factors that enhances the microbial contamination of Ready- to- eat bush meat such as water activity or availability, pH- value, redox potentials, moisture, temperature, relative humidity and nutrient content (David *et al.,* 1999; Murray, 2005; Willy *et al.,* 2007). Bush meats are commonly consumed by different people especially in Nigeria irrespective of their age and race, because of the nutritive value or nature of the meat. But they are also subjected to microbial attack and proliferation basically bacteria and fungi when not properly handled which subsequently could lead to food borne illness/ diseases among consumers due to the fact that these microbes are refers to as ubiquitous in nature, thereby causing deterioration to bush meat reducing its acceptability

and economic benefits to the humans (Willy *et al.*, 2007) When these microorganisms invade in the bush meat, it has the capacity of disfiguring the pleasant appearance of the bush meat, change the odour to that of offensive and possibly change the taste content to a soured taste which in turn would not be palatable to the consumer (Davies, 2002).Therefore, it is imperative to underscore the microbiological qualities of ready-to-eat bush meat sold at Rumuokoro market, Port Harcourt, Rivers state and its suitability for human consumption.

2. Materials and Methods

2.1 Study Area

The study was primarily based on Rumuokoro market Port Harcourt, Rivers state.

2.2 Sample Size/Collection

A total of 24 ready to eat bush meat samples were used for this study. 12 each from two species of the bush meat were collected from the same market at Rumuokoro, Port Harcourt, Rivers state. The collected samples were transferred into a sterile aluminium foil to prevent contamination and then transported Microbiology Laboratory Complex Madonna University, Elele Rivers state for bacterial analysis without further delay.

2.3 Preparation and Dilution of Sample for Analysis

The preparation and dilution of food homogenate was done using standard methods adopted by (Harrigan and MacCane, 1976). The sample was grated with grater on work bench aseptically. A gram of each sample was weighed into sterile beaker containing ten mil (10ml) of peptone water to form the stock. Six more different sterile test tube were arranged on a test tube rack and labelled in ascending order from 10-2 to 10-6 containing 9ml of peptone water for serial dilution. The stock was mixed thoroughly and 1ml was transferred into another test tube 10-2 containing 9ml of distilled or peptone water using pipette. 1ml was also transferred from the test tube 10-2 to the next and so on, till the last test tube 10-6 for serial dilution. Petri dishes were also arranged in duplicates representing the media, dilution factor used and the date of work. 1ml of each sample was dispensed into each duplicates, the dilution factor. The media were poured in respect with the label on each plate, it was gently swirled and allowed to solidify. The bacterial plates were carefully packed and incubated at 370C for 24hrs while, the fungal plate was incubated at room temperature for five days (Davies, 2002).

2.4 Enumeration of Bacterial and Fungal Count

The sample was cultured on various media using the pour plate technique. Aerobic count was determined using Nutrient agar (NA), Coliform count was determined using MacConkey agar, Escherichia coli was determined using Eosin methlylene blue agar, while Fungal count

was determined using Saboraud Dextrose Agar. The samples were incubated for 24hrs at 37oC.

2.5 Isolation of Microorganism

After the incubation, single discreet colonies from the growth media plate was sub-cultured by streaking into fresh growth media plate until pure isolates was obtained using Nutrient agar to serve as stock culture and stored in the refrigerator at 4oC.

2.6 Morphological and Cultural Examination

After the incubation, the macroscopic and microscopic characteristics of the microbial growth on various media were observed and recorded. The macroscopic observation involved examination of the colour, elevation form, margin and surface growth of the organisms. Microscopic observation involved gram staining procedure which is used to differentiate between Gram positive from negative. (Cheesbrough, 2007).

2.7 Gram Staining Technique

Gram staining was done as described by Anele *et al.(*2021) "A loopful of water was placed in a grease free sterile slide and then a portion of the organism was spread to make a smear. The smear was air dried and heat fixed. The smear was covered with crystal violet and allowed to stand for one minute, the stain was washed off and excess water was drained. The smear was covered with Gram's iodine and allowed to stand for one minute. The excess iodine was drained off and rinsed gently. 75% alcohol was also used as a decolourizer and spread on the smear until the drops coming off the slide were a pale violet colour, for 20seconds. The slide was washed gently with water. The smear was counterstained with safranin for 120 seconds. It was washed with water and the smear was allowed to blot dry. A drop of the immersion oil was placed on the smear and the slide was viewed under the microscope at the oil immersion objective. Gram positive cells appeared purple under the microscope and Gram negative cells appeared pink or red under the microscope.

2.8 Biochemical test

The biochemical test that were carried out are as follows: Indole test, Sugar fermentation test, Oxidase test, Citrate test, Catalase test, Methyl Red Voges Proskauer test (MRVP),

Motility test and Triple Sugar Iron test as described by (Cheebrough, 2007; Anele *et al.* 2021).

2.9 Statistical Analysis

The results obtained from this study were edited, coded and subjected to different statistical investigation. Mean occurrence was determined for various samples. Analysis of variance (ANOVA) was used to determine the significance at 95% internal ANOVA (Anele *et al.*, 2021).

3. RESULTS

3.1 Mean count of microorganisms associated with Ready-to- eat bush meat sold at Rumuokoro market, Port Harcourt, Rivers state

Antelope bush meat as shows in Table 1 had higher TAPC (8.09± 0.15log10CFU/g), compared to Grasscutter (7.62± 0.9log10CFU/g). CC and FC were however higher on Antelope meat (6.74± 0.17 and 4.03± 0.54log10CFU/g) compared to CC and FC on Grasscutter (6.46± 0.51 and 3.85 ± 0.47log10CFU/g), although the mean difference was statistically significant ($p<0.05$).

3.2 Percentage distribution of microorganisms isolated from dried Ready-to-eat bush meat sold at Rumuokoro market, Port Harcourt, Rivers state

The bacterial and fungal on the Ready-to- eat bush meat as indicated in Table 2 presented *Staphylococcus aureus* (26.9%), *Pseudomonas* spp (23.1%) and *Bacillus* spp (30.8%) isolates on the investigated Antelope had higher occurrence than Grasscutter samples, while *Escherichia coli* (25.0%) was higher in grasscutter than Antelope sample occurrence of *Aspergillus* spp and *Penicillium* spp were higher on Grass cutter samples (57.1%) and

(42.9%) respectively compared to the Antelope meat (55.6%) and (44.4%) respectively. There was significant difference ($P<0.005$).

Table 1: Mean count of microorganisms associated with Ready-to- eat bush meat sold at Rumuokoro market, Port Harcourt, Rivers state

Samples	TAPC	CC	EC	FC
Antelope	8.09± 0.15	6.74±0.17	5.08±1.61	4.03± 0.54
Grasscutter	7.62±0.19	6.46±0.51	4.83±1.53	3.85± 0.47

Keys:

TAPC= Total Aerobic Plate Count

CC= Coliform Count

EC= *Escherichia coli*

FC= Fungal Count

Table 2: Percentage distribution of microorganisms isolated from dried Ready-to-eat bush meat sold at Rumuokoro market, Port Harcourt, Rivers state

Isolates	Antelope	Grasscutter	Total no of isolates	Frequency of occurrence (%)
Bacteria				
Staphylococcus spp	7(26.9%)	4(25.0%)	11	28.9
Pseudomonas spp	6(23.1%)	2(12.5%)	8	21.1
Bacillus spp	8(30.8%)	3(18.8%)	11	28.9
Escherichia coli	5(19.2%)	3(25.0%)	8	21.1
Total	**26**	**12**	**38**	**100**
Fungi				
Aspergillus spp	4(57.1%)	5(55.6%)	9	52.6
Penicillium spp	3(42.9%)	4(44.4%)	7	47.4
Total	**7**	**9**	**16**	**100**

4. Discussion

From the result obtained, it was observed that microbial loads on sample obtained from Antelope were higher than the one obtained from grass cutter. This shows that the microbial quality of grass cutter is relatively better when compared to Antelope. The high microbial count in Ready – to- eat bush meat sold at Rumuokoro market in Rivers state is indication of improper cooking and handling of Ready-to-eat bush meat by producer and the retailers. Most bacteria and fungi isolated are soil pathogens and opportunistic pathogens. Similar values were obtained from (Jones *et al.*, 2011). The presence of *Staphylococcus* spp indicates that, consumers of Ready- to – eat bush meat sold at Rumuokoro market are at risk of contacting food borne intoxication. The significant difference observed in Total aerobic plate count (TAPC) in Antelope when compared to Grass cutter could be as a result of improper handling by retailers. Similar findings were recorded by (Bryan *et al.*, 1997) who also investigated some bacterial count like *E coli*, *Staphylococcus aureus*, *Bacillus* spp with yeast and mold on this meat product, and it was as a result of inadequate hygienic practice by the producers and the retailers. Furthermore, additional bacterial contamination of raw material may occur at selling site during cutting and chopping using the same material and products (Mosupye and Von Holy, 2000). In this regard, it was observed that the raw meats as well as ingredient were cutted, chooped using the same material and products. Numerous factors may be responsible for the spoilage of fresh ready- to-eat bush meat, such as conditions of evisceration, and exposure to ambient temperature and relative humilidity. Macroscopic alterations like the presence of moisture, mould, maggots and a nauseating smell are often observed on the meat at the selling point to consumers. This situation can be explained by illegal practices by salesmen, which involve injection of water into smoked bush meat or soaking of smoked bush meat in order to add volume to the dried muscle tissue. The amount of illegal, bush meat entering has increased in recent years, with the increased demand for

farmed bush meats. Consumption of bush meat can constitute a threat to public health, because some of them are not properly preserved (Malaisse, 1982). In developed world, studies of some bacteria that can be isolated from Ready- to- eat bush meat include the genus, *Lactobacillus, Acinetobacter, Bacillus, Micrococcus, Staphylococcus, Pseudomonas* and *Leuconostoe* etc. Some of the fungi that can also be isolated *Aspergillus* spp and *Penicillium* spp. Transmission of enteric pathogens to human through the consumption of contaminated food such as meat have been reported (David *et al.,* 1999). *Bacillus* spp and *Staphylococcus* spp are usually found in environment and on people's hand which could be detrimental to the bush meat and consume when not managed adequately (Oliver *et al.,* 2009).

5. Conclusion

Due to the high cost of fresh meat, some people go for Ready- to- eat bush meat as an alternative source of meat and protein. Therefore, producers and wholesalers of dried bush meat and cooked bush meat should be careful in handling and storage to avoid further contamination as consumers of these meats could be at risk of serious health challenge. Thus the users of these meats should be careful not to consume these meats uncooked.

6. Recommendations

Since the preservation of ready- to- eat bush meat is by drying in smoking, cooking or refrigerating method, hence the following recommendations should be maintained:

1. The bush meat should be preserved in such a way that it is not in direct contact with soil to avoid contamination by soil microorganism.
2. It should not be kept in a wet environment, as this could lead to microbial growth.
3. Handlers should always wash hand before and after handling as improper hand washing is the major cause of food borne illness.

4. Consumers of such meat should learn food hygiene practices such as, soaking the meat in warm salt solution, proper washing and well cooked before consumption

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