ASSESSMENT OF MICROBIAL COUNT LOADS OF BUSH MEATS SOLD AT DIFFERENT MARKETS IN BENIN CITY, EDO STATE, NIGERIA

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Abstract: -The preliminary study was carried out to assess the microbial count load of bush meat at different markets in Benin City, Edo State Nigeria. A reconnaissance survey was carried out to obtain a total of twenty (20) commissioned markets within the city, which were later structured according to their local government area. Forty (40%) sampling intensity was used to purposively select nine (9) markets where duiker, cane rat and bush pig were sold respectively from the twenty (20) markets within the metropolis. Samples of smoked bush meat were purchased from nine markets (Uselu, Use, Egor, Ekiosa, Oregbeni, Arbico, Ekiuwa, New Benin, Oliha respectively), and transported to the laboratory using sterile plastic bags. Five (5g) of each sample were accurately weighed on an electric scale. The bush meat was aseptically chopped into pieces with sterile surgical knife and the chopped bush meat was then inoculated into a nutrient broth for Twenty four (24) hours to enable fastidious organism growth. After Twenty four (24) hours, the broth containing the bush meat were sub-cultured into Mac-conkey, Blood agar, Chocolate agar, Sabrose agar and Potatoes dextrose agar. Then after inoculation, the plates were read and isolates recovered were subjected to gram staining and biochemical analysis for identification such as coagulase, catalase, indole citrate, urease and oxidase tests. Therefore, the count of microbial load of bush meat for each of the selected markets was estimated and a statistical design (RCBD) was used to analyze it. The result showed that the samples had high viable counts. Bacteria counts ranged from 1.73×10^6 to 9.46×10^6 cfu/g which is above the threshold level for delicatessen ($>6 \times 10^5$ cfu/g). Seven (7) bacteria isolates: *Staphylococcus epidermilis*, *Proteus mirabilis, Escherichia coli, Streptococcus feacalis, Staphylococcus aureus, Providencia freundii,* and *Proteus vulgaris* were identified. *Staphylococcus epidemilis* was the most common isolate followed by *Proteus mirabilis*, while Oregbeni and Uselu markets had the highest and least bacteria count respectively. The findings indicated no fungi growth in the bush meat which could be due to the preservative method. The statistical analysis showed that there was no significant difference (P>0.05) among the selected bush meat species and also among the selected markets. It could be concluded that when the preservative and hygienic procedure are not properly carried out, microbial activities increases which caused the deterioration of the bush meat. The large number of microbial counts load in this study may be attributed to the fact that there is no agency monitoring the handling and hygiene of the sales of bush meat sold at various markets in Benin City. Meat handlers and sellers should be properly educated on the adverse effect of lack of proper personal and environmental hygiene and sanitation and to ensure that the bush meats used for consumption purposes should be adequately and properly cooked and preserved before use.

Key-words: Assessment, Microbial count load, Bush meat, Deterioration, Preservation.

1. Introduction

Meat is the edible part of domestic mammals; however, recent definition of meat has a broader meaning which encompasses all mammalian species, as well as fish, shellfish, poultry, and more exotic species such as frogs and alligators [1]. Similarly, meat also refers to animal tissue used as food, mostly skeletal muscles and associated fat but it may also refer to organs, including lungs, livers, skin, brains, bone marrow, kidneys, and a variety of other internal organs as well as blood [2].

Any meat from wild animals is called bush meat and it could be consumed fresh, smoked, salted, or sun-dried. Smoked bush meat, however, is the final product most widespread and readily available in the rural, sub-urban and urban markets in most African settings [3] and [4]. The term "bush meat" stands for meat that has been sourced from wild animals and is meant for human consumption. It mainly refers to meat from Africa, but it can also be used for "wild" meat from Asia or South America. The consumption of bush meat as such is nothing new. However, the amount of meat as well as the global distribution of bush meat has increased drastically. There are various developments and changes that have contributed to the increased amount of bush meat such as population growth, more efficient hunting through more sophisticated weapons and the accessibility of formerly isolated remote forest areas.

In major cities in Nigeria, bush meat is sold in the open market exposed to the sun and dust without packaging and as such encounters microbial contamination [5]. The high demand in the urban regions means that the bush meat trade has become a lucrative business. Nowadays, regional foods are not only available at the local market anymore; they can be distributed and sold around the globe within a matter of days. What used to be a means to self-sufficiency and feeding a family has now become a global market with a growing demand. Bush meat consumption is by no means sustainable. Demand for bush meat has created a vibrant trade, which is endangering the existence of wildlife and destroying forests and grasslands, as hunters employ crude hunting methods like bush burning.

In Nigeria about 25 percent of the population relies solely on resources of animal protein from bush meat. In Ghana, up to 60 percent of the population relies on bush meat as their source of animal protein. In Tanzania and Kenya 60 percent and 35percent of their population respectively depend solely on bush meat as their source of animal protein. Therefore, wild meat has supplemented conventional source of animal protein, the indiscriminate and unsustainable use of wild animal for food as greatly affect their population, However, as human populations grow, the interactions between humans and wildlife will increase, making it possible for zoonotic transmission of diseases from animal hosts, more likely to increase [6].

The meat of wild animals (commonly called bush meat) has been the primary source of animal protein for most communities. As a result, people have developed a preferred taste for bush meat, such that it now fetches a premium over beef and other meat.. Bush meat is central to the livelihood of many poor rural dwellers that consume and trade in it [7]. Projections suggest that the take-off of wild animals from nature in Africa is six times higher than a sustainable use would be, Wildlife Conservation Society (2003), [8]. Nearly 5 million tons of bush meats are being traded in Central and West Africa [9], [10].

The aim of this study is to assess the prevalent microbial count load in smoked game meat sold at retail outlets in Benin City, Nigeria.

2. Materials and Method

2.1 Study Site

The study area is Benin metropolis, which comprises of Egor, Oredo and Ikpoba-okha local government area. The area is located between latitude 6.10N to 6.80N of the equator and longitude 5.40E to 6.00E. The elevation is 80m above sea level and occupies a land area of 923,768km^2 with a population of 3,233,366 people comprising 1,633,946 and 1,599,430 for male and female respectively (National population census N.P.C,2006). The climate of the state is tropical and is characterized by humid forests in the South and derived savanna in the North. The annual rainfall ranges 1500mm-2000mm. It has an average temperature of 25^0 c in the rainy season and

28^0 c in the dry season. The moist tropical forest dominates the states with lowland rainforest accounting for 76.5% of the total land area of the state.

2.2 Sampling methods

A reconnaissance survey was made to a total number of twenty (20) markets at the three local government areas in Benin City where bush meat were sold. Three markets were purposively selected from each local government area (Egor, Ikpoba-Okha, and Oredo) respectively.

2.3 Sample collections

Twenty-seven samples of smoked bush meat were procured from nine different selected markets (Uselu, Use, Egor, Ekiosa, Oregbeni, Arbico, Ekiuwa, New Benin, Oliha markets respectively) located at three Local Government Area. They were transported to the laboratory using sterile plastic bags with each bush meat packed individually and taken to University of Benin Teaching Hospital Medical Laboratory for analysis. The 27 samples were obtained within three days from the selected markets.

2.4 Microbial analysis

The bush meat was aseptically chopped into pieces with a sterile surgical knife. The pieces of the bush meat were inoculated into a nutrient broth for 24 hours to enable the fastidious organism to grow. After 24 hours, the broth containing the bush meat were sub-cultured into Mac-conkey, Blood agar, Chocolate agar, Sabrose agar and Potatoes dextrose agar. This was then inoculated for 24 hours at 37^0c. After incubating for 24 hours, the plates were read and isolates recovered were subjected to gram staining and biochemical analysis for identification such as coagulase, catalase, indole citrate, urease, and glucose.

2.5 Statistical analysis

The data were subjected to analysis of variance (ANOVA) and a randomized complete block design (RCBD) was used. Significant differences between treatment means were determined by Duncan's multiple range tests.

3. Results and Discussion

3.1 Identification of the bacterial organisms

Table 1 shows that *Staphylococcus epidermidis* and *Proteus mirabilis* were the most common bacteria observed, followed by *Escherichia coli* in the selected markets.

Table 1: Isolate bacteria from the bush meat

Markets	Species	Bacteria	Cell shapes
Oliha	Duiker	*Staphylococcus epidermilis*	Cocci
	Cane rat	*Escherichia coli*	Bacilli
	Bush pig	*Proteus mirabilis*	Bacilli
Ekiuwa	Duiker	*Streptococcus feacalis*	Cocci
	Cane rat	-	-
	Bush pig	-	-
New Benin	Duiker	*Staphylococcus epidermidis*	Cocci
	Cane rat	*Staphylococcus aureus*	Cocci
	Bush pig	*Proteus mirabilis*	Bacilli
Arbico	Duiker	*Staphylococcus epidermidis*	Cocci
	Cane rat	*Providencia freundii*	Bacilli
Ekiosa	Duiker	*Staphylococcus epidermidis*	Cocci
	Cane rat	*Proteus mirabilis*	Bacilli
	Bush pig	-	
Oregbeni	Duiker	*Staphylococcus epidermidis*	Cocci
	Cane rat	*Proteus mirabilis*	Bacilli
	Bush pig	*Escherichia coli*	Bacilli
Egor	Duiker	*Staphylococcus epidermidis*	Bacilli
	Cane rat	*Proteus vulgaris*	Bacilli
	Bush pig	-	-
Uselu	Duiker	*Staphylococcus epidermidis*	Cocci
	Cane rat	*Proteus mirabilis*	Bacilli
	Bush pig	-	-
Use	Duiker	*Staphylococcus epidermidis*	Cocci
	Cane rat	*Proteus mirabilis*	Bacilli
	Bush pig	-	-

3.2 Isolated bacteria load (CFU/ML)

Table 2 shows that Oregbeni market has the highest bacterial count load while Uselu market has the lowest bacterial count load and it also revealed that duiker has the highest bacterial count load while the Bush pig has the lowest bacterial count load.

Table 2: Summary of result for the isolated bacteria load (CFU/ML)

Markets	Duiker	Cane rat	Bush pig	Total	Mean
Oliha	27×10^5	25×10^5	30×10^4	5.50×10^6	1.83×10^6 [a]
Ekiuwa	23×10^5	28×10^5	23×10^4	5.33×10^6	$1.77 \times l66$ [a]
New Benin	32×10^5	22×10^5	27×10^4	5.67×10^6	1.89×10^6 [a]
Arbico	24×10^6	$27 \times 1\,0s$	24×0^4	2.67×10^7	8.96×10^6 [a]
Ekiosa	29×10^5	29×10^5	26×10^5	8.40×10^6	2.80×10^6 [a]
Oregbeni	26×10^6	20×10^4	22×10^5	2.84×10^7	$9.46 \times 1\,06$ [a]
Egor	26×10^5	29×10^5	22×10^5	7.70×10^6	2.56×10^6 [a]
Uselu	24×10^5	25×10^5	28×10^5	5.18×10^6	1.73×10^6 [a]
Use	28×10^5	28×10^5	21×10^4	5.31×10^6	1.77×10^6 [a]
Total	6.84×10^7	2.15×10^7	8.53×10^6	9.84×10^7	
Mean	7.60×10^6 [a]	2.33×10^6 [a]	9.48×10^5 [a]		

*Means with the same superscripts are not significantly different at P<0.05 for DMRT.

The result indicated in table 1 showed that *Staphylococcus epidermidis* was the most common isolate identified in the bush meat and it causes conjunctivitis and urinary infection. *Proteus mirabilis* was also a common isolate identified in the bush meat and it causes several illnesses to humans such as kidney stone. *Escherichia coli, Streptococcus feacalis, Staphylococcus aureus, Providencia freundii,* and *Proteus vulgaris* were also identified. Some of the bacteria isolate in this study were also isolated in the findings of a related study of [11], as the major causes of serious health problems. At Oliha market, *Escherichia coli* was identified which signified that the bush meat had a faecal contamination. Faecal contamination could be explained by the lack of hygiene during the preparation of smoked meat, during storage or during the preparation of smoked meat for sale. Improper smoked bush meat may carry diseases such as smallpox, chickenpox, tuberculosis, measles, yellow fever and rabies as reported in the study of [12]. Fungal growth was not available in the bush meat which may be due to the smoking technique or preservation level which removes the moisture content that may favour the growth in the meat. Staphylococci species has also been identified in the study of [13] as pathogens

that are likely to be found associated with fermented meat.

The result of the mean values in table 2 showed that the samples had high viable counts. Bacteria counts ranged from 1.73×10^6 to 9.46×10^6 cfu/g which is however passable as indicated by [14] in the standard microbial load specification in animal product, stating that total viable microbial count of less than half a million is satisfactory, half a million to less than ten million is passable while ten million and more is unsatisfactory. The values of microbial count load obtained in this study are more than those reported by [13] in a similar study. The difference might be due to disparity in the processing methods, sanitation of the processing area, handling as well as the personal hygiene of the sellers. This calls for public health awareness, improved method of processing and adequate hygiene practice as well as better sanitary handling of bush meat sold at different markets in Benin City. [15].

The result in table 2 shows that Oregbeni market has the highest bacterial count load while Uselu market has the lowest bacterial count load. Result from this study revealed that duiker (*Cephalophus maxwellii*) has the highest bacterial count load and this is in agreement with the findings of [5]. While bush pig (*Potamochoerus porcus)* has the lowest bacterial count load.

It was discovered that there was no significant difference (P>0.05) in terms of bacterial load between the markets and also between the bush meat respectively, this is in agreement with what was reported by [5] but disagrees with the findings of [16] who stated that the factors that affect microbial growth in meat is the intrinsic properties i.e. physical properties of the bush meat and its extrinsic properties i.e. the environmental factors. This finding revealed that the main factors that affected microbial growth on bush meat from some of the markets were likely due to a lack of proper smoking on the side of the meat handlers or sellers and improper hygienic and handling procedures adopted by the smoked bush meat sellers. This is in agreement with the findings of [17] who reported that lack of proper smoking and proper hygienic handling of smoked products would result in a high microbial load. [18] also conducted a related study in the suburb of Accra, Ghana and found

out that bacterial organisms were the cause of contamination and some of these organisms were also present in this study and he concluded that unhygienic practices and poor handling by butchers and sellers of meat were the major cause of contamination.

Conclusion

In major cities in Nigeria, bush meat sold in the market is exposed to the sun and dust without packaging and as a result encounters microbial contamination and lipid oxidation. The outcome of microbial count load from this study has very high viable count across the various markets in the study area which may cause serious illnesses and this call for concern among bush meat consumers. The bacterial organisms were found to be pathogenic. It was observed that the level of contamination were above the threshold level for delicatessen. The microbial count load for the various markets as well as between the bush meat was not statistically different $(P>0.05)$. Although smoking as a means of preservation increases the shelf life of the bush meat thereby reducing spoilage and helps in inhibiting the activities of microorganisms, however when not properly carried out, microbial growth and activities still continue, leading to the deterioration of the bush meat. This call for public health awareness and microbiological standards should be strictly followed in sales of bush meat which give assurance that food being consumed should not be an avenue for the spread of infectious disease of food poisoning. The processing line should be carefully monitored with appropriate quality control systems such as the principles of Good Manufacturing Practice (GMP), Total Quality Management (TQM) and Hazard Analysis and Critical Control Points (HACCP). This will ensure a safe, wholesome and qualitative product is made available to the general public for consumption.

[1]   Nakai S. Modler WH (2000). Food proteins, 1sted. Wiley-VCH, Inc., New York, U.S.A pp. 128-133.

[2]   Hammer GF (1987). Meat processing ripened products. Fleischwiritschaft 67:71-74. http://www.the onion.com/ contents/news. April 2009.

[3]   Ntiamoa-Baidu, Y ., (1997); Wildlife and food security in African FAO (Rome).

[4]   Onadeko S.A. (2004); Home On The Range: Crises, Consequences And Consolations

[4]   BCTF. 2003. BCTF Fact Sheet: Global Human Health. Bushmeat Crisis Task Force. Washington, DC. 2 pages.

[5]   Ebabhamiegbeho, P.A, Nwande C.F, and Igene J.O (2011): Lipid and Microbial Evaluation of Smoke-Dried Grasscutter on Retail. *35th Conference/AGM of the Nigerian Institute of Food Science and Technology Proceedings*.

[6]   Hogenboom, Melissa (October 18, 2014). "Ebola: Is bushmeat behind the outbreak?". BBC News. Retrieved October 21, 2014.

[7]   ACET (The African Centre for Economic Transformation) (2014); Bushmeat and the future of protein in West African.

[8]   Barnett, R. (2000) Food for Thought: The Utilisation of Wild Meat in Eastern and Southern Africa. TRAFFIC/ WWF/IUCN, Nairobi, Kenya.

[9]   Fa, J.E., C.A. Peres, and J. Meeuwig (2002) Bushmeat exploitation in tropical forests: an intercontinental comparison. Conservation Biology 16: 232-237.

[10]   Wilkie, D.S., Carpenter, J.F. (1999) Bushmeat hunting in the Congo Basin: An assessment of impacts and options for migration. Biodiversity and Conservation 8: 927-955.

[11]   Ebabhamiegbeho, P.A, Amudede, M.M, Evivie, S.E and Ekhoritomwen (2016): Assessment of microbiological status of ready-to-eat fruits and vegetable salads sold in the University of Benin Community. *Journal of Agriculture, Forestry and Fisheries (Volume 15(1), 2016.*

[12]   Mohammed M.A.M., 2012. Molecular characterization of diarrheagenic Escherichia coli isolated from meat products sold at Mansoura city, Egypt. Food Control, 25, 159-164

[13]   Zakpaa, H.D, Imbeah, C.M and Mak-Mensah, E.E, (2009): Microbial characterization of fermented meat products on some selected markets in the Kumasi metropolis, Ghana. *(African Journal of Food Science Vol 3(11) pp. 340-346).*

[14]   Wilson NRP, Dyertt EJ, Hughes BR, Jones CRV (1981): Meat and meat products, factors affecting quality control, 5th ed. Applied Science Publishers Ltd., England pp. 81-108.

[15]   Igene J.O. and Ebabhamiegbeho, P.A. (2007): The Scientific basis of Quality Assurance for Livestock Products. J. Agric Forest. And Fish. Vol. 8 (1&2).

[16]   Rombouts FM, Nout R (1994). Food Microbiology and Hygiene. Encyclope dia of Human Biology, Academic Press 3:661-665.

[17]   Abolagba, O. J. and Iyeru O. A. (1998): Study of Insect Pests Infecting Traditionally   Processed Fish Sold in Benin City Metropolis, Nigeria. Nig. J. Applied Sci; 16: 25-29.

[18]   Soyiri, IN, Agboji HK, Dongdem JT, (2008): A pilot microbial assessment of beef sold in the Ashaiman market, a   suburb   of   Accra, Ghana. Afr. J. Food   Agric.   Nutr. Dev. 8(1): 91-103.