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SURVEY OF BACTERIA AND PESTS OF SMOKED *CLARIAS GARIEPINUS* (AFRICAN CATFISH) OBTAINED FROM IKPAYONGO, ABINSI AND DAUDU MARKETS

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ABSTRACT

Survey of bacteria and pests of smoked *Clarias gariepinus* (African catfish) obtained from selected Markets (Ikpayongo, Abinsi and Daudu Markets) in Benue State Nigeria was carried out. Of the markets where the survey was carried out, microbial population count was highest in Daudu market (6.26±0.29) while the least (6.14±0.56) was recorded for Ikpayongo market. Of the 9 isolates (*Shigella sp, Staphylococcus aureus, Proteus sp, Bacillus cereus, Bacillus sp, Esherichia coli, Streptococcus sp, Micrococcus sp* and *Enterobacter sp*) while *Bacillus sp* has the highest mean population count from Abinsi market, highest mean population counts (7.75±0.25 and 7.67±0.20) were recorded for *Shigella sp* from Daudu and Ikpayongo markets, respectively. The lowest mean population counts (4.15±3.45), (4.45±0.05) and (5.75±0.25) were recorded for *Streptopcoccus sp, Staphylococcus aureus* and *Esherichia coli*, respectively.

Highest mean fungi population counts (6.82±0.63) was recorded for Daudu markets while the least (6.28±0.65) was recorded for Abinsi market. Of the six (6) fungi isolates (*Penecillium sp, Aspergillus flavus, Aspergillus sp, Mucor sp, Aspergillus fumigates,* and *Rhizopus sp*), highest mean fungi population count (8.65±0.15), (8.80±0.30) and (8.65±0.15) were recorded for *Aspergillus sp* from Abinsi, Daudu and Ikpayongo markets, respectively while the lowest (2.56±0.20), (2.64±0.03) and (2.61±0.70) mean fungi population counts were recorded for

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Rhizopus sp from Abinsi, Daudu and Ikpayongo markets, respectively. Highest mean bacterial count (6.87±0.05) was recorded for Daudu market while lowest mean bacterial count (6.27±0.01) was recorded for Ikpayongo market. Also, while highest mean fungi count (6.77±0.01) was recorded for Daudu market, the lowest (6.21±0.01) was recorded for Abinsi market. Three species of pest (*Blow flies, Necrobiarufibes* and *Dermestes maculate*) were observed during the study period. Of the three species of pest, the highest percentage (%) frequency of occurrence (12.00), (38.00%) and (55.60%) were recorded for *Blow flies*, *Necrobia rufibes* and *Necrobia rufibes* from Abinsi, Daudu and Ikpayongo markets, respectively while the lowest (11.00%), (11.00%) and (11.50%) were recorded for *Necrobia rufibes*, *Blow flies* and *Blow flies* from Abinsi, Daudu and Ikpayongo markets, respectively. Generally, *Necrobia rufibes* was most prevalent while blow flies were the least abundant pest observed.

Keywords: Bacteria, Pests, *Clarias gariepinus*, Ikpayongo, Abinsi, Daudu Markets

INTRODUCTION

Fish farming is fast becoming the bailout point of the protein need of Africans and Nigerians in particular. Thomas *et al*. (2006) and Edwin *et al*. (2019) posited that fish is the highest contributor of animal protein in Nigeria with over 34% of all the animal protein sources in Nigeria. This accounts for the leading position of Nigeria in fish production in Africa from 1999 to date both in volume and value. In this scenario, the production ecology has it that fish production from brackish water is about 0.60%, fresh water 72.3% and marine water 27.1%. Of all these production level, catfish (Clarias sp) ranks the highest of the cultured fish in Nigeria with about 33%.

In the third world, more than 70% of fish catches were smoked in fishing communities for the purpose of preservation (Ward, 2018). Smoking of fish and/or meat products is an ancient processing technique and has continued to find usefulness in modern times in Nigeria. Many fish species have very good preservation qualities after salting, sun drying and even smoking (Madu *et al.*, 2016). Preservation methods are applied with an intention to making the fish safer and extend its shelf-life (Ghazala, 2015). Fish smoking seems to be more favored method of' preservation (Akintola and Lawai, 2017). Akinola *et al*. (2018) reported that despite the rudimentary nature of process of traditional methods, lack of control over the drying rate, sometimes results to under-drying or over-drying; expose the fish to unexpected winds, dust, dirt, insect infestation, and contaminants. The shelf-life of smoked fish products depends largely on the initial bacterial contamination of the raw material; the decrease of water in the tissues due to brining and pre-drying, the activation of putrefactive micorflora due to heat treatment, the

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amount of smoke components that penetrate the product and on the temperature, air humidity and oxygen levels during storage (Sikoresski *et al*., 2017).

MATERIALS AND METHODS

Twenty one (21) samples of smoke-dried *Clarias gariepinus* comprising of 7 samples each were obtained from Ikpayongo with coordinates 7.5725^0N and 8.5968^0E, Abinsi with coordinates 7.7534^0N and 8.7522^0E, and Daudu Markets with coordinates 7.750^0N and 8.5474^0E in Gwer-East, Makurdi and Guma local government area of Benue State, Nigeria, respectively.

Inoculation of Media Plates

Using the pour plate method, a sterile micro-pipette was used to aspirate 100µ1 (0. lrnl) each of the serially diluted samples onto the various agars and labeled according to dilution and agar used. All the inoculated samples were incubated at 7°c for 24 - 48 hours after which all plates were read. Each of the plates was observed for growth and colour of colonies. Growth from the plates were subjected to further test such as Bio-chemical test, Gram staining, motility (using hanging drop method) test and coli form test to confirm their identity.

Preparation of Media

Nutrient Agar, Potato Dextrose Agar, Brilliant Green Agar and Mannitol Salt Agar were aseptically weighed, distilled water was transferred into the conical flask and the media were sterilized inside the autoclave for 15 minutes at 121˚C, allowed to cool and poured into the plates respectively. Thiosulphate Citrate, Bile Salts, Sucrose Agar and Salmonella-Shigella Agar were weighed, distilled water transferred and the media homogenized and made sterile with the use of magnetic stirrer and hotplate to boiling point for 10mins under frequent agitation and allowed to cool to about 45˚C before it was poured into the inoculated plate.

Cultivation and Enumeration of Bacteria

Fine grinded smoked catfish sample (1g) was aseptically weighed and was transferred into a MacCartney bottle containing (9 mLs) of sterile distilled water, and shaken thoroughly to make 10-1 dilution. Serial dilutions were carried out using sterile syringe that delivered the required volume 1 mL accurately to make decimal dilutions of 10-2 to 10-10. Before every 1 mL dilution transfer, the diluents were shaken well for proper mixing of the sample with the diluents. Using pour plate method, 0.1 mL of inoculums was transferred into sterile labelled Petri-dishes. About 20 mLs of sterilized molten Nutrient Agar, Potato Dextrose Agar, Brilliant Green Agar, Mannitol Salt Agar, Thiosulphate Citrate, Bile Salts, Sucrose Agar, cooled to about 45˚C, was poured into

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the inoculated Petri-dishes and allowed to gel. Some plates were also prepared as control to check on the sterility of the diluents. The plates were then incubated at 37˚C for 24 hours.

Purification of Isolates

Nutrient Agar (20 mLs) was transferred to sterile Petri-dishes and allowed to solidify after which they were dried in a hot air oven at 30˚C; this was done to get rid of moisture on the cover of the plates and on the agar itself. Suspected colonies of *Vibrio cholerae, Staphylococcus aureus, Escherichia coli, Salmonella spp* etc. were purified by streaking on Nutrient agar plates and were subjected to Gram staining and other Biochemical tests. Other microbiological activities carried out were gram staining reaction and other biochemical tests such as that of the Citrate, Kligler Iron agar, Sulphide Indole and Sugar Utilization.

Statistical Analysis

Simple percentage was used to analyze the total number of insect pests collected from the species of fish used for the research.

Results of the Study

Result of the mean microbial population counts of *C. gariepinus* from Abinsi, Daudu and kpayongo markets are presented in Table 1.

Microbial population count was highest in Daudu market (6.26±0.29) while the least (6.14±0.56) was recorded for Ikpayongo market. Of the 9 isolates, while *Bacillus sp* has the highest mean population count from Abinsi market, highest mean population counts (7.75±0.25 and 7.67±0.20) were recorded for *Shigella sp* from Daudu and Ikpayongo markets, respectively. The lowest mean population counts (4.15±3.45), (4.45±0.05) and (5.75±0.25) were recorded for *Streptopcoccus sp, Staphylococcus aureus* and *Esherichia coli,* respectively.

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Table 1: Mean microbial population counts of *C. gariepinus* from Abinsi, Daudu and Ikpayongo markets

Bacteria isolates	Markets		
	Abinsi	**Daudu**	**Ikpayongo**
Shigella sp	7.15±0.15^{a}	7.75±0.25^{e}	7.67±0.20^{de}
Staphylococcus aureus	7.35±0.20 ^{a}	4.45±0.05^{a}	7.40±0.10^{de}
Proteus sp	5.45±0.25 ^{a}	6.40±0.10^{cd}	0.00±0.00^{a}
Bacillus cereus	5.70±0.00 ^{a}	5.56±0.25^{bc}	5.65±0.25^{b}
Bacillus sp	7.85±0.35 ^{a}	7.55±0.25^{e}	7.80±0.10^{e}
Esherichia coli	5.50±0.20 ^{a}	4.65±0.65^{ab}	5.75±0.25^{b}
Streptococcus sp	4.15±3.45 ^{a}	7.15±0.26^{de}	7.50±0.20^{de}
Micrococcus sp	6.05±0.55 ^{a}	5.85±0.25^{c}	6.45±0.25^{c}
Enterobacter sp	6.90±0.20 ^{a}	6.95±0.25^{de}	7.00±0.20^{cd}
Total	**6.23±0.39**	**6.26±0.29**	**6.14±0.56**

Results of the mean fungi population counts of *C. garirpinus* from Abinsi, Daudu and Ikpayongo markets are shown in Table 2.

Highest mean fungi population counts (6.82±0.63) was recorded for Daudu markets while the least (6.28±0.65) was recorded for Abinsi market. Of the six (6) fungi isolates, highest mean fungi population count (8.65±0.15), (8.80±0.30) and (8.65±0.15) were recorded for *Aspergillus sp* from Abinsi, Daudu and Ikpayongo markets, respectively while the lowest (2.56±0.20), (2.64±0.03) and (2.61±0.70) mean fungi population counts were recorded for *Rhizopus sp* from Abinsi, Daudu and Ikpayongo markets, respectively.

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Table 2: Mean fungi population counts of *C. gariepinus* from Abuinsi, Daudu and Ikpayongo markets

Bacteria isolates	Markets		
	Abinsi	**Daudu**	**Ikpayongo**
Penecillium sp	4.70 ± 0.26^{b}	6.28 ± 0.01^{b}	6.57 ± 0.27^{b}
Aspergillus flavus	6.60 ± 0.10^{c}	7.00 ± 0.40^{b}	6.53 ± 0.18^{b}
Aspergillus sp	8.65 ± 0.15^{d}	8.80 ± 0.30^{d}	8.56 ± 0.15^{c}
Mucor sp	8.18 ± 0.28^{d}	8.66 ± 0.16^{d}	8.15 ± 0.25^{c}
Aspergillus fumigates	7.20 ± 0.20^{c}	7.50 ± 0.60^{c}	7.20 ± 0.40^{b}
Rhizopus sp	2.56 ± 0.20^{a}	2.64 ± 0.03^{a}	2.61 ± 0.70^{a}
Total	**6.28±0.65**	**6.82±0.63**	**6.60±0.59**

Results of the mean bacteria and fungi counts of *C. gariepinus* from the three markets are shown in Table 3. While highest mean bacterial count (6.87 ± 0.05) was recorded for Daudu market, lowest mean bacterial count (6.27 ± 0.01) was recorded for Ikpayongo market. Also, while highest mean fungi count (6.77 ± 0.01) was recorded for Daudu market, the lowest (6.21 ± 0.01) was recorded for Abinsi market.

Table 3: Mean bacteria and fungi counts of *C. gariepinus* from the three markets

Counts	Markets		
	Abinsi	**Daudu**	**Ikpayongo**
Bacteria	6.54 ± 0.0^{b}	6.27 ± 0.05^{a}	6.87 ± 0.01^{c}
Fungi	6.21 ± 0.01^{a}	6.77 ± 0.01^{c}	6.51 ± 0.01^{b}

Results of the percentage frequency of occurrence (%) of pest of *C. gariepinus* from the three markets are shown in figure 1.

Three species of pest were observed during the study period. Of the three species of pest, the highest percentage (%) frequency of occurrence (12.00), (38.00%) and (55.60%) were recorded for *Blow flies*, *Necrobia rufibes* and *Necrobiar ufibes* from Abinsi, Daudu and Ikpayongo markets, respectively while the lowest (11.00%), (11.00%) and (11.50%) were recorded for *Necrobia rufibes*, *Blow flies* and *Blow flies* from Abinsi, Daudu and Ikpayongo markets, respectively.

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Generally, *Necrobia rufibes* was most prevalent while blow flies were the least abundant pest observed.

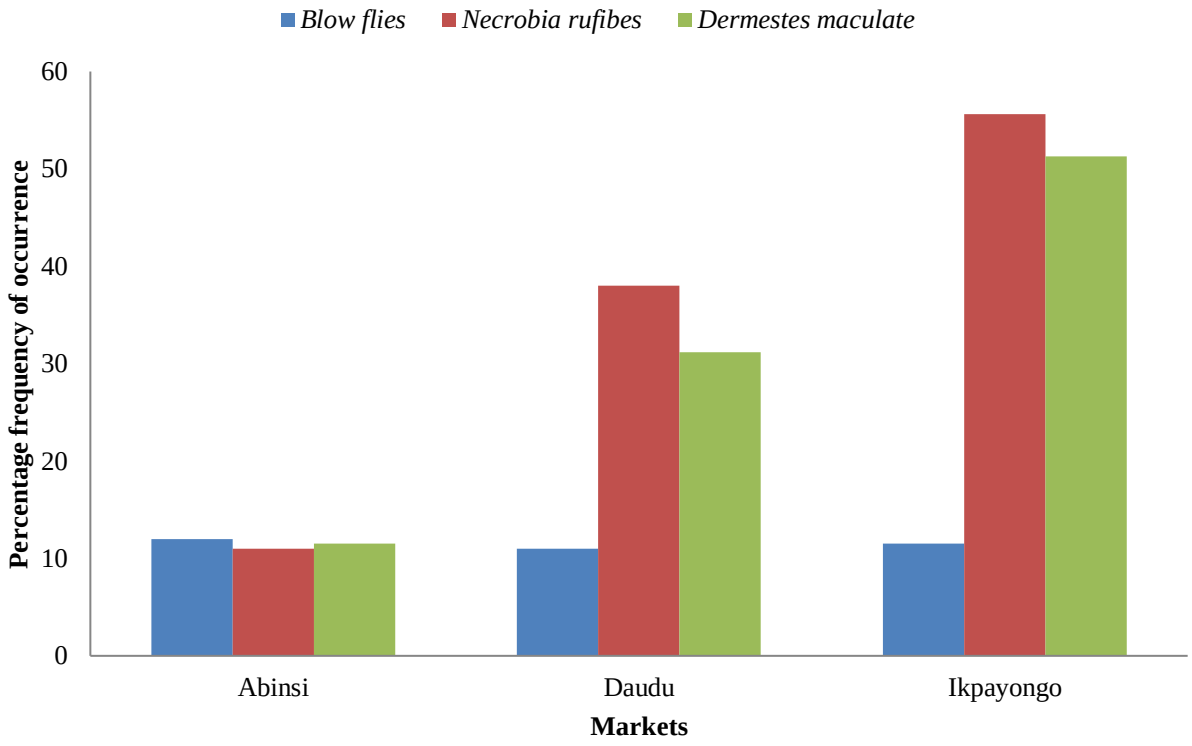

Fig. 1: Percentage frequency of Occurrence (%) of pests of *C. gariepinus* from the Abinsi, Daudu and Ikpayongo markets

DISCUSSION

In this study, evaluation of microbial qualities of smoked catfish was carried out to determine the absence and presence of the target food borne pathogens such as *Salmonella, Staphylococcus aureus*, *Escherichia coli*. The biochemical test carried out on isolates from these smoked catfish showed the presence of *Shigella sp*, *Staphylococcus aureus*, *Proteus sp*, *Bacillus cereus, Bacillus sp, Esherichia coli, Streptococcus sp, Micrococcus sp, Enterobacter sp* and *Salmonella typhi* which according to Daramola *et al*., (2020) that isolation of pathogenic and spoilage organisms such as *E. coli, Staphylococcus aureus, Listeria monocytogenes*, *Aspergillus flavus* to mention but just a few, raises public health concerns about safety in consuming smoked fish products from our markets and cause a high rate of spoilage leading to shorter shelf/storage life of the product. Meanwhile, organisms that cause food-borne diseases have been reported to include *E.coli, Bacillus* species, *Clostridium botulinum*, molds, fungi and yeast (Osakue *et al*, 2016).

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Tainting of fish with these organisms is attributed mainly to poor handling by processors and traders who expose smoked fish to unsanitary conditions. *E. coli* is often implicated in gastroenteritis associated with poor handling of food. Some of the diseases caused by these microbes are listeriosis manifesting as meningitis, abortion and pre-natal septicaemia affecting mostly immune-compromised individuals, pregnant women and infants. *E. coli* causes life threatening epidemic gastroenteritis in humans for example, travellers'diarrhoea (ETEC) also called "Delhi belly". *Bacillus cereus* produces toxins that cause a disease that is more an intoxication than a food borne infection. Also *S. aureus* is known to cause enterotoxigenicity due to the production of enterotoxin and also known to cause *Staphylococcus* food poisoning which is a major type of food intoxication (Nwachukwu and Madubuko, 2013).

Pathogenic bacteria associated with fish and fishery product can be categorized into three general groups: Bacteria (indigenous bacteria) that belong to the natural microflora of fish (*Clostridium botulinum*, pathogenic *Vibrio* spp., *Aeromonas hydrophila*). Enteric bacteria (non-indigenous bacteria) that are present due to faecal contamination (*Salmonella* spp., *Shigella sp*., pathogenic *Escherichia coli*, *Staphylococcus aureus*); and Bacterial contamination during processing, storage or preparation for consumption (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Salmonella* sp.) (Lyhs, 2019). The presence of enteric bacteria in fish and fishery product is therefore seen as a sign of poor standards of process hygiene and sanitation (Daramola *et al*., 2020).

Similarly, fish are transported in non-insulated open trucks, where both the fish and traders occupy the back of the open trucks. This post-harvest infection of smoked fish is in line with Dillon *et al* (2013), studying microbiology of smoked fish in Canada found out, that the level of micro-organisms in fish reduces with smoking but increases with storage period and during transportation. Also, fish from the processing villages do not reach the outlet markets in time, as most of the feeder roads to these areas are poor and are impassable during rainy season. This observation corroborates Poulter *et al.* (2012) findings in Zambia.

The presence of these organisms confirms microbial contamination either from poor smoking of the fish, poor personal hygiene of the processors or sellers, poor environmental conditions as well as packaging and storage of the fish. Also, the isolation of *Staphylococcus* in the smoked catfish sample may be attributed to post processing contamination and *Esherichia coli* may be as a result of poor sanitation practice or contamination from the water that the fish had been harvested.

Conclusively, fish processors should be advised to choose high quality fish products. This is because, people eat smoked fish due to the flavour and texture that the fish acquires on smoking

and they deserve to eat products of high quality. Also it is noted that the contamination of the fish samples is function of post-processing handling of smoked catfish products which might not be properly done. The smoked catfish is observed to be displayed on newspaper on a flat basket and opened for flies to perch on. The houseflies contaminate them with dirt from the surrounding environment. Some of these smoked fish products are prepared or processed poorly that if they do not reach the markets the same day, they get spoilt and cause loss to the fish processor.

It is therefore recommended that Nigerians should be educated more on the post processing handling of the smoked catfish products on how to ensure that they are well packed in well ventilated baskets and transported in proper sanitized trucks. The adoption of good processing practice and the use of controlled temperature in processing and preserving of the smoked catfish are highly recommended.

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