Microbial Load of Fresh and Smoked Fish Marketed in Benin Metropolis, Nigeria

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Abstract: Microbial load was determined on both fresh and smoked fish *Clarias gariepinus* obtained from two markets in Benin City. Six (6) fish samples were analysed. Four (4) smoked and two (2) fresh. The samples were cultured and bacterial and fungal counts were carried out. Smoked fish from markets had the highest bacteria count of 450 x 10^5 cfu/g and fungal count of 300x10^5 cfu/g. Fresh and smoked fish by researchers had lower levels of microbial load. Fresh fish had 1.35x10^5 cfu/g of bacterial count and 37.7x10^5 cfu/g of fungal count. Smoked fish by researchers had bacterial count of 37.5x10^5 cfu/g and fungal count of 15.7x10^5 cfu/g. Microorganisms identified from this study include *Baccilus* sp, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *klebsiella pneumonia* and *proteus sp*. The higher levels of microorganisms identified from smoked fish purchased from the markets can be attributed to poor fish handling and improper smoking process adopted by fish mongers.

Key words: Microbial load, *Clarias gariepinus*, smoked fish, spoilage, smoking.

INTRODUCTION

Fish is a major source of protein and its harvesting, handling, processing and distribution provide livelihood for millions of people\(^3\).

It is the most important animal protein food available in the tropics, and it represents about 14% of all animal protein on a global basis\(^1,13,15\). Fish is regarded a healthier meat option due to the high content of Long Chain Polyunsaturated Fatty acids (LCPUFAs), which are associated with improving health and preventing diseases of old age\(^17\).

In Nigeria, fish constitutes 40% of animal protein intake. In fact, Ames\(^4\) reported that fish represents a significant proportion of between 30-80% of total annual protein in the diet of consumers either as fresh or cured. Fish is a particularly important protein source in regions where livestock is relatively scarce.

Immediately fish dies, it remains in first class quality only for a short while\(^10\). However, spoilage soon sets in which is occasioned by an increase in the ambient temperature that triggers favourable conditions for microorganisms to thrive. Other factors are the Poikilothermic nature of fish, a high postmortem pH in the flesh, the presence of non-protein nitrogen (NPN) in large quantities and the presence of trimethylamine oxide (TMAO)\(^20\).

The Poikilothermic nature of fish selects for bacteria that can thrive in a wide range of temperatures. The presence of NPN and TMAO on decomposition of fish flesh, give rise to the off-flavours typically found in spoilt fish.

The autolytic enzymes such as protease all act on the fish muscle causing softening of fish flesh and off-flavours\(^31\). These enzymes result in the breakdown of Adenosine Triphosphate (ATP) to Adenosine Diphosphate (ADP), Adenosine Monophosphate (AMP), hypoxanthine and others amines. Microorganisms such as bacteria also act on the dead fish, producing volatile bases such as Trimethylamine (TMA) and Hydrogen Sulphide (H\(_2\)S). Oxidation of lipids is associated with a decrease in triacylglycerols and phospholipids and an increase in fatty acids and often result in a product with off flavor (rancid) which may not be appealing to many consumers\(^9\).

The smoking of fish from smouldering wood for its preservation dates back to civilization\(^24,13,12\). It is also noted that apart from giving the product a desirable taste and odour, smoking provides a longer shelf-life through its anti-bacterial and its oxidative effects lowering pH, imparting colouration as well as accelerating the drying process and acting as antagonist to spoilage agents\(^1,24,27,16\).

Fish is thus a product that needs proper handling and processing in order to preserve nutrients and its functional components that promote good health. Okonta and Ekelemu\(^22\) conducted a preliminary study on the microorganisms associated with smoked fish and reported *E.coli* and *Staphylococcus aureus* as the predominant microorganisms infecting fish spoilage in Asaba area of Nigeria.

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MATERIALS AND METHODS

Collection of Samples: Two (2) fresh and one (1) smoked Clarias each was collected from two markets in Benin metropolis namely, Santana and Ogida markets in Oredo and Eker Local Government Areas respectively. The average weight of the fresh fish was 750g. Of the two fish one from each of the market was smoked by the researchers, using the Chorkor Smoker in the department of Fisheries, University of Benin, Nigeria. A total of six (6) fish were used for the study (two fresh/live, and four smoked). The samples were then transported in clear sterile plastic bags to the laboratory.

Culture Media Preparation and Sterilization: Nutrient Agar (NA) and Sabourand Dextrose Agar (SDA) were used. In preparing the media, 28 g of NA was dispersed in 1 litre of ionized water, but 250ml each was needed; so 7g of NA and 15.5g of SDA were used. The weighed media were mixed with the water in a conical flask, autoclaved for 15mins at 121°C and used. The weighed media were mixed with the water in a conical flask, autoclaved for 15mins at 121°C and left to cool. The top of the conical flask was wrapped with foil to prevent contamination. The area (bench) where the work was done was properly cleaned with disinfectant soap and water, wire loop was flamed before and after use.

Preparation of Sample for Culture /Serial Dilution: A ten-fold serial dilution was made. For each fish sample, four (4) test tubes were used for the serial dilution. The test tubes were filled with 9ml of deionized water. For the fresh fish, swab stick was used to swab the body (external part), then the fish was cut open and the accessory breathing organs, gills and intestine (internal parts) were used for the experiment. For the smoked fish, the tail and the skin were used for the external part, while the intestine, gills and eggs were used for the internal part. 1g of these samples were transferred into the assigned test-tube (making it 10ml) and thoroughly mixed. Further sequential dilutions were made by taking 1ml of 10ml mixture to other test-tubes.

Culturing, Incubating, Colony Count and Identification: After the serial dilution, 1ml of each sample, taken from the 3rd and 4th test-tubes were transferred to a petri-dish that has been appropriately labeled with marker. The pour plate method was used for culture. The petri-dish was shaken in an anti-clockwise direction to enable the agar that was poured into it set and spread out evenly.

The plates of bacterial count were kept in the incubator at 37°C for 24hours while that of fungi were left on the bench for 48hrs, at room temperature. All the Petri-dishes were incubated upside down, after 24 hours, the bacterial count was done. Colonies appeared as clusters and each plate was counted and recorded. Same was done for fungi plates after 48 hours. Identification was carried out using standard procedure and biochemical tests such as the gram staining techniques, catalase test, oxidase test were according to Wikipedia. The study showed that the predominance microorganisms affecting smoked fish from the markets had the highest microbial load (bacteria and fungi) when compared with smoked fish by the researcher (Table 1). Furthermore, Table 3 gives a vivid account of the higher load of microbes from smoked fish obtained from the markets. This can be attributed to contamination by microorganisms in the surrounding environment during and after the smoking process or the inefficiency of the smoking process.

Discussion: The microorganisms isolated and identified from the fresh fish samples can be said to be normal flora of the fish. The normal microbial flora of the fish are not initially harmful, as they even help in preventing the invasion of the fish flesh by other microorganisms but they become pathogenic when there is an enabling environment that promotes their growth. Bad handling which can lead to bruises, poor hygiene and delayed processing and preservation of the fish after harvest. The study showed the internal parts harbored more microorganisms than the external part, confirming the observation of Hasen and Olsaten. Pseudomonas aeruginosa was identified as the most dominant microorganism in all the fish samples studied. It is a rod-shaped bacteria which forms pink colonies when grown on blood agar. It is found in the skin flora and most environments according to Wikipedia. The study showed Staphylococcus as the second most isolated organism after Pseudomonas and was found to be the most dominant in the smoked fish samples. This is a confirmation of the work of Okonta and Ekelemu who reported Staphylococcus as one of the predominant microorganisms affecting smoked fish and causing their spoilage. Klebsiella pneumoniae was identified in the already smoked smoked fish from the

RESULTS AND DISCUSSION

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markets and absent in the smoked fish by researchers and the fresh fish. It is a among the most common gram-negative bacteria, belonging to the group called the coliforms. Infections such as pneumonia and respiratory tract diseases result in the host with low immunity. When isolated and grown on MacConkey agar, it forms pink-to-red colonies, signifying that it is a lactase-fermenting bacteria\textsuperscript{[26]}.

\textbf{Bacillus} was present in virtually all fish samples tested (smoked and fresh). It is a gram-positive, obligate aerobe rod shaped, endospore forming bacteria\textsuperscript{[29]}. Two \textit{Bacillus sp.} are considered medically significant; \textit{B. anthracis} which causes anthrax and \textit{B. coagulase} also causes food spoilage. Colonially, they are large, spreading and irregularly shaped. When viewed under microscope, they appear as rods with a bulge which contains the endospore\textsuperscript{[19]}.

The study equally showed \textit{Proteus sp.}, like \textit{klebsiella} was only present in the already smoked fish. \textit{Proteus sp.} are members of the normal intestinal flora. They are aerobic, gram-negative rod, and oxidase negative bacteria\textsuperscript{[5]}.

The microorganisms in this study are similar to those reported by\textsuperscript{[22,6,7,2]}. All the pathogens isolated are of food and public health implication and hence hazardous and injurious to human health if consumed. The Isolation of \textit{Staphylococcus aureus} in this work is of practical impact. It is an evidence of poor sanitary condition and lack of or inadequate packaging of the products as they are always exposed at the markets\textsuperscript{[21]}.

\textbf{Conclusion:} The study showed that though smoking 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 fish. High occurrence of microorganisms was recorded in the already-smoked fish from the two markets sampled. The smoked fish by the researchers nevertheless recorded low occurrence of microorganisms due to good sanitary conditions observed prior to and during the smoking process which is generally lacking in the smoked fish from the markets.

\textbf{Table 1: Microbial Count Obtained from fresh and smoked fish from two markets in Benin City.}

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Market</th>
<th>Type of colony</th>
<th>Part of fish</th>
<th>No of colonies (cfu x 10\textsuperscript{5})</th>
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<td>External</td>
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<td>Fungi</td>
<td>15.0</td>
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<td></td>
<td></td>
<td>External</td>
<td>50.0</td>
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<td>Santana</td>
<td>Bacteria</td>
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<td>External</td>
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<td>Bacteria</td>
<td>Internal</td>
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<td>Bacteria</td>
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<td>External</td>
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<tr>
<th>Treatment</th>
<th>Market</th>
<th>Type of Colony</th>
<th>Part of Fish</th>
<th>Identified Microorganisms isolated</th>
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</thead>
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<td>Smoked fish</td>
<td>Ogida Bacteria</td>
<td>Internal</td>
<td>P. aeruginosa</td>
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<td></td>
<td></td>
<td></td>
<td>Fungi</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Internal</td>
<td>Mucor, Absidia</td>
<td></td>
</tr>
<tr>
<td>Smoked fish</td>
<td>Santana Bacteria</td>
<td>External</td>
<td>Proteus sp., Staphylococcus aureus</td>
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<td></td>
<td></td>
<td>Internal</td>
<td>Proteus sp., Bacillus sp. and P. aeruginosa</td>
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<td>Fungi</td>
<td>Mucor</td>
<td></td>
</tr>
<tr>
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<td>Ogida Bacteria</td>
<td>External</td>
<td>Proteus sp., Bacillus sp. and S. aureus</td>
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<td>Smoked fish</td>
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<td>Klebsiella sp., Proteus sp. and P. aeruginosa</td>
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<td>Mucor</td>
<td></td>
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<tr>
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<td>External</td>
<td>Bacillus sp. and S. aureus</td>
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<tr>
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<td>Internal</td>
<td>Bacillus sp. and S. aureus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fungi</td>
<td>External</td>
<td>Mucor</td>
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<td>Mucor</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Bacteria</td>
<td>Internal</td>
<td>P. aeruginosa and S. aureus</td>
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<td></td>
<td>Fungi</td>
<td>Mucor</td>
<td></td>
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<td>Smoked fish by</td>
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<td>External</td>
<td>Bacillus sp. and S. aureus</td>
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<td>Researchers</td>
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<td>Fungi</td>
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<td>Internal</td>
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<tr>
<td>Santana</td>
<td>Bacteria</td>
<td>Internal</td>
<td>P. aeruginosa</td>
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<td></td>
<td></td>
<td>Fungi</td>
<td>Mucor</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Internal</td>
<td>Mucor</td>
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</table>

Table 2: Microorganisms isolated and identified from two markets in Benin City

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Market</th>
<th>Type of Colony</th>
<th>Part of Fish</th>
<th>Identified Microorganisms isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoked fish</td>
<td>Ogida Bacteria</td>
<td>Internal</td>
<td>P. aeruginosa</td>
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<td></td>
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<td>Mucor, Absidia</td>
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<tr>
<td>Smoked fish</td>
<td>Santana Bacteria</td>
<td>External</td>
<td>Proteus sp., Staphylococcus aureus</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Internal</td>
<td>Proteus sp., Bacillus sp. and P. aeruginosa</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Fungi</td>
<td>Mucor</td>
<td></td>
</tr>
<tr>
<td>Already</td>
<td>Ogida Bacteria</td>
<td>External</td>
<td>Proteus sp., Bacillus sp. and S. aureus</td>
<td></td>
</tr>
<tr>
<td>Smoked fish</td>
<td>Santana Bacteria</td>
<td>Internal</td>
<td>Klebsiella sp., Proteus sp. and P. aeruginosa</td>
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</tr>
<tr>
<td></td>
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<td>Mucor</td>
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</tr>
<tr>
<td>Smoked fish by</td>
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<td>External</td>
<td>Bacillus sp. and S. aureus</td>
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<tr>
<td>Researchers</td>
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<td>Internal</td>
<td>Bacillus sp. and S. aureus</td>
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<td>Santana</td>
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</tr>
<tr>
<td></td>
<td>Internal</td>
<td>Mucor</td>
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</tbody>
</table>

Table 3: Effects of Treatments on Microbial Count (cfu x 10^6 )

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoked fish by Researchers</td>
<td>1.97b</td>
</tr>
<tr>
<td>Fresh fish</td>
<td>5.78b</td>
</tr>
<tr>
<td>Smoked fish from market</td>
<td>17.1a</td>
</tr>
</tbody>
</table>

NB: Means with same letters are not significantly different while those of different letters are significantly different.
Microbial count from fresh fish were at considerable safe levels. However, considering the public health implications of the poor bacteriological and microbiological state of the smoked fish, particular attention should be paid by the processors and fish mongers to their safety through proper processing, storage and handling procedures.

The spoilage of fish and fish products depends on a number of factors. These factors as well as these spoilage mechanisms must be thoroughly understood before developing proper handling and pretreatment methods and preservation technologies for fresh and smoked fish, in addition to proper packaging and storage of the products.

REFERENCES


