

EVALUATION OF MICROBIAL LOAD IN SOME SMOKED MARINE FISH PRODUCTS SOLD IN OPEN MARKETS IN NNEWI METROPOLIS, NNEWI NORTH LOCAL GOVERNMENT AREA OF ANAMBRA STATE, NIGERIA

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Abstract

In Nigeria, people's general awareness of food safety has grown tremendously in recent years. In order to determine the safety of the fish for consumption, this study evaluated some microbiological quality of smoked marine fishes consumed around Nnewi City, Nnewi North Local Government Area of Anambra State, Nigeria. The research was carried out in four markets, where smoked fish were analysed to find out how many bacteria were present in them. Herring (*Clupea harengus*, "Sawa"), Horse Mackerel (*Trachurus trachurus*, "Kote"), Great Barracuda (*Sphyraena barracuda*, "Panla"), and Atlantic Mackerel (*Scomber scombrus*, "Titus") were the chosen smoked fish samples that were tested. Fish samples were taken from open market places that were on display in different markets. The acquired results suggested that some types of bacteria were present in the fish samples under examination. Across all samples, the average total aerobic bacterial counts varied from 2.5×10^3 cfu/g to 7.3×10^3 cfu/g. The average coliform counts from the smoked fish were: 4.7×10^3 cfu/g for *Clupea harengus*; 5.1×10^3 cfu/g, for *Scomber scombrus*; and 3.7×10^3 cfu/g for *Sphyraena barracuda*. However, the smoked fish samples that were chosen had an average *Salmonella* count (cfu/g) that varied from 1.3×10^3 cfu/g to 3.5×10^3 cfu/g. It is advised that users of smoked fish cook it to a suitable temperature in order to prevent food-borne illnesses in the study area, given the levels of microbial load found in the fish samples that were analysed.

Keywords: Fish products, Microbial load, Markets, Food safety

INTRODUCTION

Fish is a highly perishable product because of its high susceptibility to autolysis, oxidation and hydrolysis of fats and microbial spoilage, hence, fish smoking is one of the traditional processing methods aimed at preventing or reducing postharvest losses [1]. Additionally, Bankole *et al.* [2] reported that heat application removes water and inhibits bacterial and enzymatic actions in fish. Fish can be preserved after harvest using freezing (which works best when the fish is conditioned to -10°C) and refrigeration (4°C). Other commonly used techniques include smoke and sun drying, all of which have been linked to increased germicidal effect with rising temperatures. One of the most significant animal proteins found in the tropics is fish, which accounts for 14% of all animal proteins worldwide [3]. The health benefits of eating fish are driving up demand for fish consumption in Nigeria. Other factors contributing to this trend include the country's growing population, and the ongoing drought, which are lowering the cost and availability of red meat (cattle, sheep, and goats) [4]. Fish must be handled carefully and promptly after being caught in order to maintain its freshness because it is a highly perishable food [5]. Since eating has a direct impact on health, it is crucial for food inspectors, manufacturers, and handlers to keep food free of harmful microorganisms, particularly when it comes to fast food or other items that are intended to be consumed raw. It has been established that a variety of microbes can alter the nutritional value of food, posing a health risk to those who consume contaminated food. It has been demonstrated that a variety of locally sold food items are significantly contaminated with *Staphylococcus*, *Bacillus*, and other types of bacteria [6].

Human foodborne diseases and poisoning can be brought on by microorganisms. Larger populations of bacteria physically present in the consumed food can contribute to some cases of food poisoning. Apart from bacterial infections, viral infections can also result in gastroenteritis, which manifests as low-grade fever, nausea, vomiting, diarrhea, abdominal discomfort, and paralysis. In new-borns and people with weakened immune systems, *Listeria monocytogenes*, a bacterium from infected animals, causes septicaemic aborting [7]. According to recent estimates, people are consuming more fish as a result of consuming less meat due to the high cost of meat. It is predicted that the demand for fish has increased by 25% in Nigeria recently. Actually, all of our foods have a natural population of microbes, which may have the chance to proliferate and become more numerous during processing due to contamination from the environment, utensils, or worker clothing. The majority of food-poisoning substances only manifest symptoms when consumed in large doses following food multiplication. Contamination-related illnesses are among the most common health issues and, as a result, a major contributor to lower economic output [8]. In addition to being palatable and filling, fish and fish products are generally less expensive than other animal protein sources. They are also great providers of sufficient amounts of nutrients found in food, such as minerals, vitamins, lipids, proteins, and fats. They are a rich source of vital nutrients that are needed to complement diets for both adults and infants. Tryptophan, methionine, and lysine—the three amino acids that are deficient in plant-based protein—are particularly abundant in fish protein [9]. When the bones of fish are consumed, mineral components like zinc, phosphorus, iron, and calcium are present. Vitamins A and D, as well as riboflavin, are all found in fish. These nutrients also support the growth of microorganisms [10].

The unsanitary surroundings in which fish are left after being caught and before being served has drawn attention from all across the world [11]. Furthermore, it is probable that inadequate cleanliness measures play a role in the microbiological contamination of fish. The contamination of fish with pathogens is a major problem for human health, safety, and the environment [12, 13]. Consumption of fish infected with diseases has caused serious health concerns in Nigeria and other parts of the world, and has been linked to several reported deaths [14]. However, according to Cartoni *et al.* [15], fish's qualities make them an ideal medium for bacteria to live in and proliferate. Because bacteria like *Vibrio* spp. and *E. coli* are the primary drivers of food-borne diseases, their presence in fish has caused scientists great concern [16, 17]. The majority of people also thought that eating smoked salmon without further heating processing was really safe. For this reason, it is not unusual to see individuals consuming fish at the market without first heating it after smoking. Smoked fish is typically sold in developing countries without consideration for the environmental pollution caused by microbes. In Nigeria, smoked fish products may contain bacteria from market centres and processing facilities before they are consumed by consumers. This is because many hawkers and processors typically exhibit their products in an open manner, which may act as a possible source of microbial contamination. Because of this, the study evaluated the potential microorganisms linked to smoked fish in order to determine the extent of their implications for public health in the study area. Thus, the purpose of this study was to evaluate the microbiological quality of smoked fish consumed in the vicinity of Nnewi, Anambra State, Nigeria, in order to determine the fish's safety for ingestion.

MATERIALS AND METHODS

Study Area

The study was carried out in four markets namely: Eke Amaobi, Oye Agbor, Nwafor Uruagu, and Nkwo Nnewi located in Nnewi metropolis, Nnewi North Local Government Area of Anambra State, Nigeria

Samples Collection

A total of 16 samples of four types of selected smoked fish such as Herring *Clupea harengus* (Sawa). Horse mackerel- *Trachurus trachurus* (Kote). Atlantic mackerel- *Scomber scombrus* (Titus), and *Sphyraena barracuda* (Panla) were purchased randomly from Eke Amaobi, Oye Agbor, Nwafor Uruagu, and Nkwo Nnewi markets in Nnewi Metropolis of Anambra State. Each sample was transported to the laboratory in a sterile polythene bags for analysis.

Preparation of Media

The media used are Nutrient agar (N.A.), Shigella Agar (SSA), Eosin Methylene Blue Agar (EMB Agar). All media used were prepared according to the manufacturers' instructions. The mean counts of bacteria in colony forming units per gram of samples were determined [18].

Preparation of Cultures

The samples were serially diluted after maceration under aseptic conditions. The appropriate dilutions were inoculated on the different agar media. All cultures were incubated in duplicate at 37°C for 24 – 48 hours. The bacteria were inoculated on Nutrient Agar for 24- 48 hours, Salmonella - Shigella Agar (SSA) for 24 hours, and coliform on Eosin Methylene Blue (EMB) agar for 24 hours. Colonies on plates containing 30 - 306 colonies were counted and multiplied by the dilution factor [19].

Preparation of Samples and Enumeration of Microorganisms

The fish samples were surface sterilized separately in 3.5% sodium hypochlorite solution (w/v) with constant agitation for 7 minutes, rinsed thoroughly with sterile distilled water until the traces of hypochlorite were removed and were then dried in an oven at 45°C for 24 hours. The muscles and of the fish samples were pulverized separately using a blender (maker). Five milliliters were taken from each sample into a sterile bottle containing 450 ml of sterile peptone physiological saline to form a stock culture [20]. The sample bottles were placed on a rotator shaker at 120 RPM for 1 hour. 10-fold dilutions were subsequently prepared with peptone physiological saline. Aerobic mesophilic bacteria were enumerated on plate count agar (PCA, Oxoid) at 37°C for 24 hours. Coliform count were enumerated on de Man, Rogosa and Sharpe Agar (Merck) and incubated anaerobically at 30°C for 48 hours. Presumptive LAB was confirmed by oxidase and catalase tests, and confirmed counts were reported as lactic acid bacteria (LAB). Salmonella were enumerated on Violet Red Bile Glucose at 37°C for 24 hours while Staphylococci were counted on mannitol salt agar (Oxoid) at 30°C for 48 hours [21].

Identification of Bacteria

The isolates were identified using a number of characteristics [21]. Their cultural and morphological characteristics were of vital importance in this process and were thus observed. Motility tests as well as biochemical test were also carried out. Inocula were aseptically transferred from each slide into plates of respective media using a streak plate technique. The isolates were purified by repeated streaking on their respective media. Bacterial plates were incubated at 37°C for 24 hours while fungal plates at 25°C for 72 hours. A 24 hour old culture was prepared from each plate for identification purposes. Bacteria isolates were identified based on their cultural characteristics, Gram staining reaction and various identification tests. Isolates were identified according to the methods described by Leroi, [22].

RESULTS

Microbiological Evaluations of some Smoked Fish Species Sampled from Some Markets at Nnewi Metropolis

The Total aerobic bacteria count (cfu/g) of selected smoked fish samples from some markets at Nnewi metropolis are presented in Table 1. The results obtained indicated that the highest values of Total aerobic bacteria count (cfu/g) value of 8.6×10^3 was observed in the specie *S.scrombus* sampled from Oye Agbor market. While the lowest value of 1.1×10^3 was observed in the specie *T. trachurus* from Nwafor Uruagu market. However, the average values of 6.3×10^3 , 7.3×10^3 , 5.4×10^3 and 2.5×10^3 were observed in *C.harengus*, *S. scrombus*, *S.barracuda* and *T. trachurus* respectively. The Coliform count (cfu/g) of selected smoked fish samples from some markets at Nnewi metropolis are presented in Table.2. The results obtained indicated that the highest values of Coliform count (cfu/g) value of 6.5×10^3 was observed in the specie *S. scrombus* sampled from Oye Agbor market. While the lowest value of 1.0×10^3 was observed in the specie *T. trachurus* from Nwafor Uruagu market. However, the average values of 4.7×10^3 , 5.1×10^3 , 3.7×10^3 and 1.4×10^3 were observed in *C. harengus*, *S. scrombus*, *S. barracuda* and *T. trachurus* respectively. The Salmonella count (cfu/g) of selected smoked fish samples from some markets at Nnewi metropolis are presented in Table 3. The results obtained indicated that the highest values of *Salmonella* count (cfu/g) 3.6×10^3 was observed in the specie *S. scrombus* sampled from Oye Agbor market. While the lowest value of 1.0×10^3 was observed in the specie *T. trachurus* from Nwafor Uruagu market. However, the average values of 2.3×10^3 , 2.0×10^3 , 3.2×10^3 and 1.5×10^3 were observed in *C. harengus*, *S. scrombus*, *S. barracuda* and *T.trachurus* respectively.

Microbial Occurrence in some Smoked Fish Species Sampled from Some Markets at Nnewi Metropolis

The microbial occurrence in some smoked fish species sampled from some markets at Nnewi metropolis are presented in Figure 1. Among the three bacteria under consideration, the total aerobic bacteria count consistently recorded higher values in all the sampled fish species under consideration, this was closely followed by Coliform count, while salmonella occurred the lowest in all the sampled fishes. The three microbes were relatively high in all the smoked fish samples. However, lower values were observed in the specie *T.trachurus*.

Table 1: Total Aerobic Bacteria Count (cfu/g) of Selected Smoked Fish Samples from Some Markets at Nnnewi

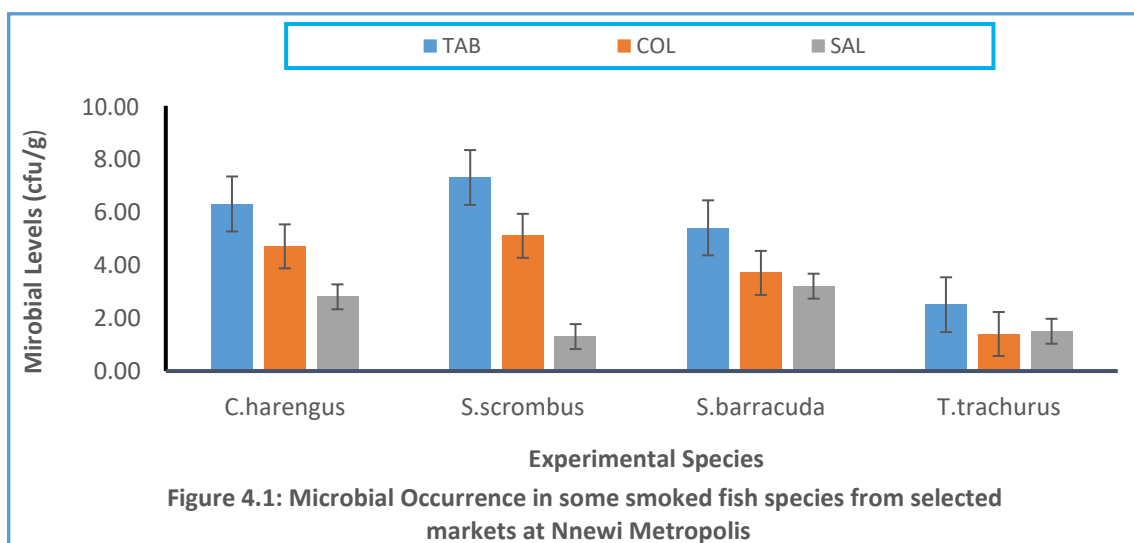
Markets	Smoked Fish Species			
	<i>C.harengus</i>	<i>S.scrombus</i>	<i>S.barracuda</i>	<i>T.trachurus</i>
Eke Amaobi	6.3x10 ³	7.3x10 ³	4.4x10 ³	3.2x10 ³
Oye Agbor	7.4x10 ³	8.6x10 ³	5.6x10 ³	1.2x10 ³
Nwafor Uruagu	5.3x10 ³	5.6x10 ³	3.3x10 ³	1.1x10 ³
Nkwo Nnewi	6.0 x10 ³	7.5 x10 ³	8.1 x10 ³	4.3 x10 ³
Average	6.3 x10³	7.3 x10³	5.4 x10³	2.5 x10³

Table 2: Coliform Count (cfu/g) of Selected Smoked Fish Samples from Some Markets at Nnnewi

Markets	Smoked Fish Species			
	<i>C.harengus</i>	<i>S.scrombus</i>	<i>S.barracuda</i>	<i>T.trachurus</i>
Eke Amaobi	4.1x10 ³	5.7x10 ³	2.6x10 ³	2.2x10 ³
Oye Agbor	6.1x10 ³	6.5x10 ³	5.5x10 ³	1.1x10 ³
Nwafor Uruagu	3.7x10 ³	3.1x10 ³	2.3x10 ³	1.0x10 ³
Nkwo Nnewi	4.8 x10 ³	5.1 x10 ³	4.4 x10 ³	1.1 x10 ³
Average	4.7 x10³	5.1 x10³	3.7 x10³	1.4 x10³

Table 3: Salmonella Count (cfu/g) of Selected Smoked Fish Samples from Some Markets at Nnnewi

Markets	Smoked Fish Species			
	<i>C.harengus</i>	<i>S.scrombus</i>	<i>S.barracuda</i>	<i>T.trachurus</i>
Eke Amaobi	1.1x10 ³	2.0x10 ³	3.0x10 ³	2.6x10 ³
Oye Agbor	1.6x10 ³	3.6x10 ³	3.8x10 ³	1.1x10 ³
Nwafor Uruagu	3.2x10 ³	1.1x10 ³	3.7x10 ³	1.0x10 ³
Nkwo Nnewi	3.1 x10 ³	1.1 x10 ³	2.4 x10 ³	1.1 x10 ³
Average	2.3 x10³	2.0 x10³	3.2 x10³	1.5 x10³



Key: TAB- The Total aerobic bacteria count (cfu/g)

COL- Coliform Count (cfu/g)

SAL- Salmonella Count (cfu/g)

DISCUSSION

The present investigation's findings regarding the higher values of microbial load in sampled fish from the study area are consistent with the findings of Abolagba *et al.* [10], who reported a similar trend in smoked *C. gariepinus* in some markets in Benin City. The higher microbial load observed could be attributed to handling practices used after processing, which greatly contributed to the contamination of the fish products; observations at the processing environment also suggest that post-processing contaminations could originate from the processing environment and extend to the retail market. Typically, processors did not wash their hands before removing the smoked fish from the oven [23]. Other processors provided assistance when removing fish from the oven, but they didn't think to wash their hands or change their clothes. Furthermore, the placement of smoked fish on trays near bare ground may raise the risk of post-smoking microbial contamination. Not only may physical dangers like sand transmit physical threats into the fish, but they could also promote contamination with soil microorganisms. Since the fish are not given any significant care before being sold, such as cleaning, sorting, and packaging, there is a problem with food safety [24].

For good bacteriological quality, the colony counts obtained are greater than the maximum count of 1 x 10³ cfu/g of fish. The fish samples had high moisture contents, which promotes the growth of bacteria. Food handlers' disregard for

fundamental hygienic guidelines could be the cause of the comparatively high quantity of coliform. High levels of bacteria were found in the smoked fish, which included *Clupea harengus* "Sawa," *Scomber scombrus* "Titus," *C. harengus* "Kote," and *Sphyraena barracuda* "Panla" from various locations. This finding is consistent with a report by Okonko *et al.* [25], who noted a comparable trend in some smoked fish in a few markets in the city of Ibadan. This can be because these foods with an intermediate moisture level have a relatively high moisture content. Coliform organisms are a type of indicator organism that suggests a potential faecal contamination [26]. The acceptable limit of bacterial contamination is 1000 cfu/g, however the amount of coliform present is higher [27]. The findings displayed in Tables 1, 2, and 3 indicated that certain foods may cause gastrointestinal problems and diarrhoea in both adults and children. The majority of these foods are stored at room temperature for extended periods of time, which can promote the growth of harmful bacterial species and cause food intoxication [28].

Salmonella sp. was examined in this study using the fish sample, as indicated in Table 3. Fish samples from the Oye Agbor market are more contaminated than those from other areas; the highest frequency of isolates from the samples came from *Scomber scombrus*, while *Trachurus trachurus* had the lowest frequency. Additionally, the majority of the organisms identified in this study may have entered these foods through contaminated open market conditions, utensil and wrapping material water, improper product storage, handling conditions that were not hygienic, and other sources. Fish from Oye Agbor and Nkwo Nnewi have the largest microbiological load; this could be because of the local environment and/or the heavy traffic in the area, which could lead to dust contamination of the fish [29]. Among the microbiological risks associated with smoked fish and shellfish products are *Salmonella* spp., *Clostridium botulinum*, and *Listeria monocytogenes*, according to Bernadi *et al.* [30].

Additionally, Omojowo and Ihuahi [6] discovered that gram-positive bacteria, potential pathogens, coagulase-positive *Staphylococcus*, and *Escherichia coli* dominated smoked fish samples from four local markets in the Minna and Kainji Lake area of Nigeria. Furthermore, Lyhs *et al.* [31] reported that the most prevalent microorganism linked to smoked fish were bacteria, including *Staphylococcus aureus*, *Proteus*, *Bacillus*, *Micrococcus*, coliform, and salmonella. These results imply that the majority of the ready-to-eat fish available in the research region market could be potential sources of food poisoning bacteria because of their high coliform count, which can lead to diarrhea and other health risks for the general public. Gastritis, which is characterized by vomiting, abdominal pain, paralysis, and low grade fever, can be brought on by the spread of *Salmonella* and other pathogens [32]. When organisms like *Bacillus*, *Escherichia coli*, *Salmonella* Sp, and so forth are prevalent and are known to be linked to infections or food poisoning, it is especially important for food handlers, consumers, and processors to maintain and improve good hygiene practices [33].

CONCLUSION AND RECOMMENDATIONS

This study shows that all the four in the study areas smoked fish markets had smoked fish samples with bacterial counts higher than the international microbiological standard's suggested limits on bacterial contamination in food, making them microbiologically undesirable. The results of the study demonstrated that although smoking can assist to restrict the activities of microorganisms, if this process is not done correctly, microbial growth and activities still occur, which causes the fish to deteriorate. Microorganisms were found in high concentrations in the pre-smoked fish from all four of the markets that were analysed. The fish should therefore not be eaten until it has been fully smoked. The following are suggested in light of the study's findings: It is necessary to implement regular hygienic procedures to prevent these germs from contaminating food items. Because high concentrations of *Coliforms*, *Bacillus* sp. and other dangerous bacteria can result in serious diseases and food poisoning, it is important to consider them more than just food contamination. Therefore, the microbiological standard of the roadside market's operations should always receive more attention. Food handlers are required to follow basic sanitary regulations while handling smoked fish because there are numerous health risks associated with direct human intake.

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