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Microbiological Quality and Some Heavy Metals Analysis of Smoked Fish Sold in Benin City, Edo State, Nigeria

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Abstract: The microbiological qualities and heavy metals (Fe, Cu, Zn, Al and Pb) levels of five smoked fish species (*Scomber scombrus*, *Tachurus trachurus*, *Pseudolithus croaker*, *Ethmallosa fimbriata* and *Clarias gariepinus*) sold in Benin City, Edo State, Nigeria were examined. Standard aerobic pour-plate techniques were used for microbial enumeration while heavy metal concentrations in fish were determined using atomic absorption spectrophotometer (AAS). Mean bacteria count of 1.24 $x10^6$ cfu/g was observed in *Scomber scombrus* while *Ethmallosa Fimbriata* had mean bacterial count of 1.85$x10^6$ cfu/g and a mean fungal count of 2.56$x10^6$ cfu/g and *Clarias gariepinus* had mean bacterial count of 1.24$x10^6$ cfu/g. *Ethmallosa fimbriata* had a lead concentration of 0.05± 0.02 mg/kg while *Clarias gariepinus* had a lead concentration of 0.07±0.01 mg/kg. *Pseudolithus croaker* and *Ethmallosa fimbriata* had copper concentrations of 0.68± 0.00 and 0.64±0.01 mg/kg respectively. *Clarias gariepinus* had iron concentration of 20.88±0.10 mg/kg while *Tachurus trachurus* had iron concentration of 18.16±0.14 mg/kg. In conclusion, caution should be exercised in consuming smoked-fish shaded openly in markets as they contain bacteria and fungi, reheating may be necessary to destroy microbial cells.

Key words: Bacteria Count % Lead % Copper % Iron % Smoked Fishes

INTRODUCTION

Fish is a high-protein, low-fat food that provides a range of health benefits. They have rich source of essential nutrients required for supplementing both infant and adult diets [1]. Today, fish has become the main supply of protein besides meat and poultry products and contributes a large percentage of dietary protein globally because of its high essential fatty acids (EFAs) known as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [2, 3].

In many countries including Nigeria, industrial wastes, agricultural runoffs, burning of fossil fuels, animal and human excretions, geologic weathering, domestic waste, geochemical structure and mining of metals have created a potential source of heavy metals pollution in the aquatic environment. Discharge of heavy metals into river or any aquatic environment can change both aquatic species diversity and ecosystems due to their toxicity and accumulative behavior [4, 5]. Aquatic organisms such as fish and shell fish accumulate metals to concentrations many times higher than present in water or sediment [6, 7]. They can take up different metals and then concentrate them in their different body organs and tissues [8]. At low levels, some heavy metals such as copper, cobalt, zinc, iron and manganese are essential for enzymatic activity and many biological processes [9], while other metals such as cadmium, mercury and lead have no known essential role in living organisms and are toxic even at low concentrations. The essential metals can also become toxic at high concentrations [10].

Fish is also susceptible to microbial contamination as millions of bacteria are present in the surface slime, gills and in the intestines of live fish [11]. Bacterial growth and invasion of fish are prevented by the body's natural defense system during life but after death, the defense system breaks down and the bacteria multiply and invade the flesh. Fish Smoking is one of the traditional processing methods aimed at preventing or reducing postharvest losses. It involves heat application to remove water and inhibits bacterial and enzymatic actions of fish [12]. Apart from giving the fish a desirable taste and

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odour, smoking provides a longer shelf-life through its anti-bacterial and oxidative effect, imparting desirable coloration as well as accelerating the drying process and acting as antagonist to spoilage [11]. This work therefore seeks to analyze heavy metals (iron, copper, zinc, aluminum and lead) and microbial contamination in five smoked fish species.

MATERIALS AND METHODS

Sample Collection: Five different smoked fish species namely *Tachurus trachurus* (Sese), *Scomber scombrus* (Mackerel), *Clarias gariepinus* (Catfish), *Ethmallosa Fimbriata* (Bonga fish) and *Pseudolithus croaker* (Meluza) were purchased from Oba market and Yanga market in Benin City, Edo State, Nigeria. The samples were wrapped in newly purchased polyethylene bags, labeled accordingly and transported to laboratory for microbiological and heavy metals analysis.

Bacteria Analysis: Sterile forceps was used to cut fish samples from the left side of the fish. 1.0 g of the fish samples was weighed out using the top loading balance, blended and suspended in 10 ml sterile water to make a stock suspension. Then, serial dilutions were carried out on each sample and then 1 ml of selected dilutions was plated using the pour plate method [13]. Enumeration of total aerobic viable count was done using Nutrient agar. All cultures were incubated in duplicate at 37°C for 24 hours. All media used were prepared according to the manufacturers' instructions. The mean count of bacteria in colonies forming units per gram of sample was determined and their means and standard error calculated using the methods of David *et al*. [14].

Isolation of Fungi: One milliliter (1ml) of selected dilutions was transferred to Sabouraud dextrose agar amended with streptomycin to inhibit bacteria growth. All cultures were incubated in duplicate at 25°C for 72 hours. After which observed colonies were subculture to obtain pure cultures which were subsequently identified using morphological characteristics, spore formation, the production of fruiting bodies and biochemical reactions.

Heavy Metal Analysis: The fish samples were homogenized separately in a mortar and weighed accurately in a porcelain crucible. Before ashing, 1ml of concentrated HNO_3 was added to the samples and allowed to pre-digest overnight in order to reduce losses of volatile metals. The samples were charred on an electric hot plate before ashing in a muffle furnace at 550°C for 4hrs. The white ash was dissolved in 5 ml of 1:1 HCl and a solution made in a 50 ml standard flask [15]. Metal concentrations of the samples were read against appropriate blank and standard solutions using a Perkin-Elmer model 306 Atomic Absorption spectrophotometer (AAS). A blank solution for the biotic samples was made by diluting 1ml concentrated HNO_3 with 5ml 1:1 HCl and a 50 ml solution made up with distilled water. The individual metals was expressed on a dry weight basis as mg/kg and the data generated were analyzed statistically.

RESULTS AND DISCUSSIONS

Highest mean bacteria count of $1.24x10^6$ cfu/g was observed in *Scomber scombrus* (Table 1) while a fungal count of $4.98x10^6$ cfu/g was recorded for *Tachurus trachurus*. *Ethmallosa Fimbriata* had mean bacterial count of $1.85x10^6$ cfu/g and a mean fungal count of 2.56×10^6 cfu/g while *Clarias gariepinus* had mean bacterial count of $1.24x10^6$ cfu/g and a mean fungal count of 1.72×10^6 cfu/g. The variations in microbial counts of smoked fish samples from markets may be due to lack of proper smoking on the side of the fish processors or/and improper hygienic and handling procedures adopted by the smoked fish sellers and buyers. This is in agreement with the findings of Abolagba and Iyeru, [16] who reported that lack of proper smoking and proper hygienic handling of smoked fish products would result in a varying microbial load.

The species of fungi isolated were identified as *Aspergillus niger*, yeast sp. and *Penicillium* sp. (Table 2). The number of fungi isolated from fish ranged from two to five. The prevalent fungus was *Aspergillus niger* having a 60% occurrence while *Penicillium* sp. had a 30% occurrence. The species of bacteria isolated were identified as *proteus* sp., *staphylococcus aureus*, *Pseudomonas* sp., *Streptococcus* sp. and *Bacillus* sp. (Table 2). *Proteus* sp. had the highest frequency of 80% while *Streptococcus* sp. and *Bacillus* sp. had a percentage frequency of 20% and 60% respectively.

The diversity of microorganisms associated with these smoked fish could be attributed to exposure at market as the tissues of fish are capable of reabsorbing moisture from the atmosphere, hence its ability to support the growth of microorganisms. Also the unhygienic nature of the market, handling and improper smoking of the fish can also contribute to the type of microorganisms seen in smoked fish. All these microorganisms isolated in this study are of public health implication and hence hazardous to human health when consumed.

Table 1: Mean microbial counts (cfu/g) of smoked fish samples

Fish Species	Total Aerobic count	Total Fungal count
A	1.58 x 10^6±0.21	4.98 x 10^6±0.04
B	2.08 x 10^6±0.21	3.00 x 10^6±0.30
C	1.24 x 10^6±0.21	1.72 x 10^6±1.00
D	1.85 x 10^6±0.21	2.56 x 10^6±0.09
E	1.38 x 10^6±0.21	2.20 x 10^6±0.40

A: *Tachurus trachurus* B: *Scomber scombrus* C: *Clarias gariepinus* D: *Ethmallosa fimbriata* E: *Pseudolithus croaker*

Table 2: Frequency distribution of microbial isolates from smoked fish samples

Microorganism	Isolate	Percentage Frequency (100%)
Bacteria	*Proteus* sp.	80
	Staphylococcus aureus	70
	Pseudomonas sp.	40
	Streptococcus sp.	20
	Bacillus sp.	60
Fungi	*Yeast*	50
	Aspergillus niger	60
	Penicillium sp.	30

Table 3: Heavy metals concentration (mg/kg) in various species of smoked fish

Fish Species	Iron (Fe)	Zinc (Zn)	Copper (Cu)	Lead (Pb)	Aluminum (Al)
A	17.28±0.02	6.80±0.01	0.34±0.02	0.02±0.00	5.93±0.02
B	18.16±0.14	5.49±0.01	0.15±0.01	0.01±0.03	4.54±0.03
C	22.47±0.09	8.89±0.08	0.68±0.00	0.29±0.01	3.97±0.01
D	30.95±0.01	14.05±0.11	0.64±0.01	0.05± 0.02	8.20±0.02
E	20.88±0.10	6.57±0.02	0.84± 0.05	0.07±0.01	4.40±0.01
FAO Limit	-	30.0 mg/kg	3 mg/kg	0.3 mg/kg	-

A: *Scomber scombrus* (Mackerel), B: *Tachurus trachurus* (Sese), C: *Pseudolithus croaker* (Meluza), D: *Ethmallosa fimbriata* (Bonga fish), E: *Clarias gariepinus* (Catfish)

The occurrence of *Staphylococcus aureus*, yeast, *Penicillium sp* and *Aspergillus niger* in the smoked fish samples were in accordance with Martin, [17] when he reported that these organisms were the commonest microorganisms associated with smoked fish. Also, similar results were also reported by Abolagba and Igbinevbo [18] and Adebayo-Tayo *et al*. [19] in smoked fish sold in Benin and Uyo metropolis respectively.

Table 3 shows the levels of Fe, Zn, Cu, Al and Pb that were expressed in mg/kg dry weight sample. The data clearly shows variation in the level of heavy metals among the smoked fish species sampled. The analysis of the selected metals in the present study revealed an order of Fe >Zn>Al>Cu>Pb in almost all the species. Accumulation of metal in different species is the function of their respective membrane permeability and enzyme system [20].

Zinc in smoked fish samples accumulated in the order *Ethmallosa fimbriata* > *Pseudolithus croaker* > *Scomber scombrus* > *Clarias gariepinus* > *Tachurus trachurus*. Zinc is one of the essential elements as copper and cobalt for both animals and humans. The varying concentration of zinc in the muscles of the fish samples could be due to the presence of large numbers of fishing vessels and trawlers which use galvanized metal coatings to prevent rusting and this ultimately find its way into the ambient media through leaching [20]. Zinc has been reported to be necessary for embryo development in fish [21].

Lead concentrations ranged between 0.01±0.03 and 0.29±0.01 mg/kg (Table 3). *Ethmallosa fimbriata* had a lead concentration of 0.05±0.02 while *Clarias gariepinus* had a lead concentration of 0.07±0.01 mg/kg. Higher concentration of lead is known to inhibit active transport mechanisms involving ATP and may also suppress cellular oxidation-reduction reactions and even inhibit protein synthesis [22]. The level of lead from this study could not be said to pose any health risk since the values were within the FAO permissible limit of 0.3 mg/kg.

Copper concentrations ranged from 0.15±0.01 to 0.84±0.05 mg/kg (Table 3). *Scomber scombrus* had copper concentration of 0.34±0.02 mg/kg while *Pseudolithus croaker* and *Ethmallosa fimbriata* had copper concentrations of 0.68±0.00 and 0.64±0.01 mg/kg respectively. The copper concentrations were similar to other studies of Rejomon *et al*. [23]; Olowu *et al*. [24] and Yilmaz, [25]. Values obtained for Cu in this study in

muscles were below the 3.0 and 1.0-3.0 mg/kg prescribed limits for food fish by WHO, [26] and FEPA, [27] for Cu in food and fish respectively, thus indicating that the fishes examined were free from Cu related toxicity.

The concentrations of iron ranged from 17.28±0.02 mg/kg to 30.95±0.01 mg/kg. *Clarias gariepinus* had iron concentration of 20.88±0.10 mg/kg while *Tachurus trachurus* had iron concentration of 18.16±0.14 mg/kg (Table 3). Fe is an essential element in human diet and fish contains relatively high amounts of readily absorbable haem iron which is better absorbed than non-haem iron. Iron forms part of hemoglobin which allows oxygen to be carried from the lungs to the tissues.

In view of the low levels of heavy metals got from the smoked fish samples analyzed in this study, constant evaluation should be carried out periodically in order to ascertain when the levels of heavy metals is above the acceptable limit for safe consumption as these metals could be passed to humans and predispose the consumers to possible health hazards. Safe disposal of domestic wastes and industrial effluents should be practiced and where possible recycled to avoid these metals and other contaminants from going into the environment. Caution should be exercised in consuming smoke-fish shaded openly because such fish could contain microbial cells and reheating may be necessary to destroy or inactivate such cells.

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