

A Comparative Analysis of the Microbial Load of Smoke-Dried Fishes (*Ethmalosa Fimbriata* and *Pseudotolithus Elongatus*) Sold in Oba and Koko Markets in Edo and Delta States, Nigeria at Different Seasons

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Abstract: A comparative analysis of the microbial load of smoke-dried fishes (*Ethmalosa fimbriata* and *Pseudotolithus elongatus*) sold in Oba and Koko markets in Edo and Delta States at different seasons was carried out. Fish samples were analysed using potato dextrose agar and nutrient agar for fungi and bacteria load of the smoke-dried fish samples. The genera of isolates identified includes; *Aspergillus*, *Mucor*, *Saccharomyces*, *Rhizopus*, *Penicillium*, *Neurospora*, *Cercospora*, *Candida*, *Trichoderma*, *Proteus*, *Bacillus*, *Micrococcus*, *Staphylococcus*, *Serratia* and *Enterococcus*. The mean total microbial load was highest for bacteria 1.1×10^7 cfu/g when compared with that of fungi 2.5×10^6 cfu/g. The mean total bacteria count was significantly higher for the rainy season 1.2×10^7 cfu/g than that of the dry season 9.2×10^6 cfu/g. While the fungi count 2.5×10^6 cfu/g were the same in both season. The mean total bacteria count of bonga from Koko 1.3×10^7 cfu/g was higher when compared to that from Benin 6.4×10^6 cfu/g for both seasons. On the other hand, the mean total fungi count of bonga from both seasons was higher in Benin 2.8×10^6 cfu/g (for dry) when compared to that from Koko 2.0×10^6 cfu/g (for rainy). The frequency of occurrence of fungi species was higher with the fungi than the bacteria for example; *Aspergillus niger*, *Mucor sp*, *Saccharomyces sp*, 4(13.3%) were the most frequently isolated organisms from all the fish samples during the dry and rainy seasons.

Key words: Microbial load, *Ethmalosa fimbriata*, *Pseudotolithus elongatus*, Smoked fishes, Oba and Koko markets.

INTRODUCTION

Fish is one of the best source of proteins, vitamins and minerals and are essential nutrients required for supplementing both infant and adult diets (Abdullahi *et al.*, 2001). In Nigeria, fish is eaten fresh, preserved or processed (smoked) and form a much-cherished delicacy that cuts across socio-economic, age, religious and educational barriers (Adebayo-Tayo *et al.*, 2008).

Fish is soft and easily damaged, therefore rough handling and bruising results in contamination of fish flesh. Fish will become unfit for human consumption within about one day of capture, unless it is subjected to some form of processing or preservation. Even after the fish has been processed, particularly if traditional methods have been used, the fish is still subject to many forms of loss and spoilage.

The microbial flora associated with freshly harvested fish is principally a function of the environment in which the fish are caught and not of the fish species; hence, the indigenous microbial populations of fish can vary significantly (Shewan, 1977).

Fish, because of their soft tissues and aquatic environment are extremely susceptible to microbial contamination. Millions of bacteria, many of them potential spoilers, are present in the surface slime, on the gills and in the intestines of live fish, although the flesh itself is normally sterile. Bacterial growth and invasion on the 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.

Microbial action has been known to play a large part in the spoilage of fish (Eyo, 2001). Bacterial spoilage is characterized by softening of the muscle tissue and the production of slime and offensive odours (Geoff *et al.*, 1991).

Fish Smoking is one of the traditional fish processing methods aimed at preventing or reducing post-harvest losses. Smoking involves heat application to remove water and it inhibits bacterial and enzymatic actions of fish (Kumolu-Johnson *et al.*, 2009).

Abolagba and Melle, (2008), Olorok, (2007), Sengor *et al.*, (2004), Eyo, (2001) Horner, (1997), Clucas and Ward, (1996) noted that apart from giving the product a desirable taste and odour, smoking provides a longer shelf-life through its anti-bacterial and oxidative effect, lowering of pH, imparting desirable colouration as well as accelerating the drying process and acting as antagonist to spoilage.

MATERIALS AND METHODS

Collection of Samples; A total of eight (8) fish samples were used. For the first analysis, one non-mouldy smoke-dried bonga and one non-mouldy smoke-dried croaker were purchased from Oba and Koko markets in Edo and Delta States. The fish samples were tied separately in sterilized polyethylene bags and taken to the laboratory for microbial analysis. The same quantity of fish samples and procedure was also used for the second analysis. The first analysis was carried out in the rainy season while the second in the dry season.

Culture Media:

Nutrient Agar (NA) was used for the culture of bacteria while Potato Dextrose Agar (PDA) was used for fungi. In preparing the media, 14g of NA was dispersed in 1litre of sterile water and 39g of PDA in 1litre of sterile water. Sterilization of the media was done with both media in a conical flask and sealed with foil paper to prevent contamination and then sterilised in the autoclave at 121°C for 15minutes after which it was allowed to cool. The media was shaken vigorously before use (Taylor *et al.*,1998).

Isolation of Microbial Isolates:

The standard aerobic pour plate count technique was used which is based on the assumption that each viable cell will yield a colony forming unit per gram of sample (cfu/gm). The aerobic colony counts of the fish sample were done in accordance with Peter *et al.*, (1992) by the pour-plate method in the following procedure;

Sterile knife was used to cut fish samples from the left side of the fish. 1.0gm of the fish samples was weighed out using the top loading balance, blended and suspended in 10ml sterile water to make a stock suspension. 1.0ml of the stock, was transferred by pipetting to a 9.0ml diluent of sterile water. This process was repeated for six other test tubes to make 10^{-7} dilution. 1.0ml of the last test tube was discarded in order to achieve equal dilution.

For Bacteria; Total count of heterotrophic bacteria was determined using nutrient agar (NA) by pour-plate technique. Aliquots of 1.0ml of the 10^{-1} , 10^{-3} , and 10^{-5} dilutions of each of the fish stock dilutions of the samples were inoculated in sterilized petri dishes in duplicates and 0.2ml of antifungal mixture was added to discourage fungi growth. Cooled molten nutrient agar was poured into inoculated plates and swirled gently to mix and solidify. Plates were incubated at room temperature for 24hours in inverted position to prevent condensation. The mean counts 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.*, (1997).

For Fungi; Aliquot of 1.0ml of the 10^{-1} , 10^{-3} and 10^{-5} dilutions of the fish stock mixture were inoculated into potato dextrose agar (PDA) in duplicates amended with antibiotic mixture to discourage bacteria growth. Plates were inoculated at room temperature for 72hours. Developing colonies were counted and mean standard error calculated using the methods of David *et al.*, (1997).

RESULTS AND DISCUSSION

Tables 1-7 shows the results obtained from the study of the comparative analysis of smoke-dried bonga and croaker fish that was carried out in which the total heterotrophic microbial count was studied in the rainy and the dry season between two fish species from two different locations. Table 1 shows the total heterotrophic bacteria count in colony forming units per gram of fish sample for rainy season. Table 2 shows the total heterotrophic fungi count in colony forming units per gram of fish sample for rainy season. Table 4 shows the total heterotrophic bacteria count in colony forming units per gram of fish sample for dry season and table 5 shows the total heterotrophic fungi count in colony forming unit per gram of fish samples. Table 7 shows the mean count in colony forming units per gram of bacteria and fungi isolated from fish samples obtained from Koko and Oba markets at different seasons.

The percentage frequency of occurrence of each microbial isolate was also determined with table 3 showing the frequency of occurrence of microbial isolates from fish samples obtained from both markets during the rainy season and table 6 showing that for dry season.

Discussion:

In this study, the mean total microbial count in colony forming unit per gram of the fish sample was highest for bacteria when compared with that of fungi. This can be as a result of the fish samples being contaminated before being processed. Also the mean total bacteria count for the rainy season was higher than that of the dry season, this may be due to high moisture content absorbed during the rainy season because of the humid atmosphere. The fungal count was the same for both seasons. The mean total bacteria count of bonga from Koko was higher than that from Benin for both seasons.

Whereas the mean total bacteria count in the dry season for the croaker in Benin was higher than that from Koko. On the other hand, the mean total fungi count of bonga from both seasons was higher in Benin, than that of Koko. Whereas in the croaker Benin had the highest rainy season than Koko dry season (see table 7).

The variations in microbial counts of fish samples from different markets and seasons in which some have higher microbial counts may be likely due to a lack of proper smoking on the side of the fish processors or/and improper hygienic and handling procedures adopted by the smoked fish sellers. This is in agreement with the findings of Abolagba and Iyeru (1998) who reported that lack of proper smoking and proper hygienic handling of smoked fish products would result in a very high microbial load.

A total of 30 isolates were obtained and identified as bacteria, fungi and yeast. The bacteria isolates which includes *Proteus sp*, *Bacillus sp*, *Micrococcus sp*, *Staphylococcus aureus*, *Serratia sp* and *Enterococcus sp* was isolated on nutrient agar.

The fungi isolates were identified as *Aspergillus niger*, *Mucor sp*, *Saccharomyces sp*, *Rhizopus sp*, *Penicillium italicum*, *Neurospora sp*, *Cercospora sp*, *Candida sp*, and *Trichoderma sp*. Tables 3 and 6 shows the frequency of occurrence of the organism isolated from the two different species (bonga and croaker) from two different locations (Benin and Koko) in Edo and Delta States of South-South Nigeria.

The highest number of isolates from the fish was obtained from the dry season fish samples than the rainy season fish samples which constituted 30 and 29% of the isolates.

The frequency of occurrence of fungi species was higher with the fungi than the bacteria for example; *Aspergillus niger*, *Mucor sp*, *Saccharomyces sp*, (4(13.3%)) were the most frequently isolated organisms from all the fish samples during the dry and the rainy seasons. This was followed by *Penicillium italicum*, *Neurospora sp*, (2(6.7%)), and *Yeast sp*, *Trichoderma sp*, *Cercospora sp*, *Penicillium sp* and *Rhizopus sp* (1(3.30%)). While *Staphylococcus aureus*, *Bacillus sp*, *Proteus sp* (4(13.3%)) where the most frequently isolated bacteria followed by *Micrococcus sp*, *Serratia sp*, *Enterococcus sp* (1(3.3%)) which where the least frequent bacteria isolates implicated in this study (see table 3 and 6). All these microorganisms isolated in this study are of public health implication and hence hazardous and injurious to human health if consumed.

The occurrence of bacteria *Staphylococcus aureus*, yeast *Saccharomyces sp* and moulds *Penicillium sp* and *Aspergillus niger* in the smoked-dried fish samples were in accordance with Martin (1994) when he stated that these organisms were the commonest microorganisms associated with smoked fish and these microorganisms were also reported by Abolagba *et al.*, (2010) in smoked fish (*Clarias sp*) sold in Benin metropolis. The presence of *Staphylococcus aureus* in fish samples according to Okonko *et al.*, (2008) might have been through handling.

The occurrence of *Aspergillus sp*, *Rhizopus sp*, and *Penicillium sp* could be due to the fact that during storage, the fish sample reabsorbed moisture from the environment which then supported the growth of the microorganisms in addition to the contamination during processing, handling and display on the market stalls (Christianah *et al.*, 2010).

Serratia sp which occurred only in the croaker from Koko during the rainy season and in the bonga from Benin during the dry season can be said to present due to contamination of the water in which the fish WAS caught from or when freshly harvested fish is kept on contaminated soil because according to Seyit *et al* (1999) and Jessica (1997), species of *Serratia* genus exist in normal microbial flora of soil and water. The presence of *Trichoderma sp* can also be as a result of the fish been kept on contaminated soil because according to Wikipedia (2011) *Trichoderma sp* are mainly isolated from forest and soils. *Proteus sp* occurrence may also be due to contamination of soil and water.

Candida sp which was found only in the bonga from Koko can be as a result of contamination from the environment or where the fish is stored and mainly where females urinate because *Candida* is common in the urinary tract of females.

Table 1: Total Heterotrophic Bacteria Count per Gram of Fish Samples (For Rainy Season).

Fish samples	Dilutions in triplicate	Dilution factors	Numbers of colony forming units/ plate ($\times 10^5$)	Average number of colonies per plate. ($\times 10^5$) $\bar{x} \pm S \bar{x}$	Organisms /gm of fish tissue
(1) Bonga (Koko market)	10^{-5}	10^5	120	120 ± 4.6	$\frac{120 \times 10^5}{1} = 1.2 \times 10^7$
	10^{-5}	10^5	112		$\frac{112 \times 10^5}{1} = 1.1 \times 10^7$
	10^{-5}	10^5	128		$\frac{128 \times 10^5}{1} = 1.3 \times 10^7$
(2) Bonga (Oba market)	10^{-5}			97.3 ± 8.1	
	10^{-5}	10^5	112		
	10^{-5}	10^5	96		$\frac{96 \times 10^5}{1} = 9.6 \times 10^6$
(3) Croaker (Koko market)	10^{-5}	10^5	136	136 ± 4.6	$\frac{84 \times 10^5}{1} = 8.4 \times 10^6$
	10^{-5}	10^5	144		$\frac{136 \times 10^5}{1} = 1.4 \times 10^7$
	10^{-5}	10^5	128		$\frac{128 \times 10^5}{1} = 1.3 \times 10^7$
(4) Croaker (Oba market)	10^{-5}	10^5	144	142.7 ± 12.7	$\frac{144 \times 10^5}{1} = 1.4 \times 10^7$
	10^{-5}	10^5	120		$\frac{120 \times 10^5}{1} = 1.2 \times 10^7$
	10^{-5}	10^5	164		$\frac{164 \times 10^5}{1} = 1.6 \times 10^7$

Table 2: Total Heterotrophic Fungi Counts per Gram of Fish Samples (For Rainy Season).

Fish samples	Dilutions in triplicate	Dilution factors	Numbers of colony forming units/ plate ($\times 10^5$)	Average number of colonies per plate. ($\times 10^5$) $\bar{x} \pm S \bar{x}$	Organisms /gm of fish tissue
(1) Bonga (Koko market)	10^{-5}	10^5	15	16.0 ± 0.58	$\frac{15 \times 10^5}{1} = 1.5 \times 10^5$
	10^{-5}	10^5	16		$\frac{16 \times 10^5}{1} = 1.6 \times 10^5$
	10^{-5}	10^5	17		$\frac{17 \times 10^5}{1} = 1.7 \times 10^5$
(2) Bonga (Oba market)	10^{-5}	10^5	20	20.0 ± 1.45	$\frac{20 \times 10^5}{1} = 2.0 \times 10^5$
	10^{-5}	10^5	23		$\frac{237 \times 10^5}{1} = 2.3 \times 10^5$
	10^{-5}	10^5	18		$\frac{18 \times 10^5}{1} = 1.8 \times 10^5$
(3) Croaker (Koko market)	10^{-5}	10^5	30	27.3 ± 1.45	$\frac{30 \times 10^5}{1} = 3.0 \times 10^5$
	10^{-5}	10^5	25		$\frac{25 \times 10^5}{1} = 2.5 \times 10^5$
	10^{-5}	10^5	27		$\frac{27 \times 10^5}{1} = 2.7 \times 10^5$
(4) Croaker (Oba market)	10^{-5}	10^5	40	37.0 ± 1.76	$\frac{40 \times 10^5}{1} = 4.0 \times 10^5$
	10^{-5}	10^5	38		$\frac{38 \times 10^5}{1} = 3.8 \times 10^5$
	10^{-5}	10^5	34		$\frac{34 \times 10^5}{1} = 3.4 \times 10^5$

Table 3: Frequency of Occurrence of Microbial Isolates from Fish Samples Obtained from Both Markets (For Rainy Season).

Isolates	Fish samples				
	No. (%)	1	2	3	4
Fungi					
Aspergillus niger	4 (13.3)	✓	✓	✓	✓
Saccharomyces_sp.	4 (13.3)	✓	✓	✓	✓
Candida sp.	1 (3.30)	✓	X	X	X
Penicillium italicum	1 (1.30)	✓	X	X	X
Mucor sp.	4 (13.3)	✓	✓	✓	✓
Yeast (orange)	1 (3.30)	X	✓	X	X
Trichoderma sp.	1 (3.30)	✓	X	X	X
Cercospora sp.	1 (3.30)	X	X	X	✓
Bacteria					
Proteus sp.	4 (13.3)	✓	✓	✓	✓
Staphylococcus_aureus	4 (13.3)	✓	✓	✓	✓
Bacillus sp.	4 (13.3)	✓	✓	✓	✓
Serratia_sp.	1 (3.30)	X	X	✓	X
Total	30	9	7	7	7
Over all total	30 (100)	29 (96.7)			

✓ - Present

x- Not present.

Table 4: Total Heterotrophic Bacteria Count in Colony Forming Units per Gram of Fish Samples (For Dry Season).

Fish samples	Dilutions in triplicate	Dilution factors	Numbers of colony forming units/ place (x10 ⁵)	Average number of colonies per plate. (x10 ⁵)	Organisms /gm of fish tissue
(1) Bonga	10 ⁻⁵	10 ⁵	184		$\frac{184 \times 10^5}{1} = 1.8 \times 10^5$
(Koko market)	10 ⁻⁵	10 ⁵	90	135.3 ± 27.2	$\frac{90 \times 10^5}{1} = 9.0 \times 10^5$
	10 ⁻⁵	10 ⁵	132		$\frac{132 \times 10^5}{1} = 1.3 \times 10^7$
(2) Bonga	10 ⁻⁵	10 ⁵	108		$\frac{108 \times 10^5}{1} = 1.2 \times 10^7$
(Oba market)	10 ⁻⁵	10 ⁵	55	53.5 ± 30.9	$\frac{55 \times 10^5}{1} = 5.5 \times 10^6$
	10 ⁻⁵	10 ⁵	162		$\frac{162 \times 10^6}{1} = 1.6 \times 10^6$
(3) Croaker	10 ⁻⁵	10 ⁵	100		$\frac{100 \times 10^5}{1} = 10.0 \times 10^7$
(Koko market)	10 ⁻⁵	10 ⁵	52		$\frac{52 \times 10^5}{1} = 5.2 \times 10^6$
	10 ⁻⁵	10 ⁵	76	76.0 ± 13.9	$\frac{76 \times 10^5}{1} = 7.6 \times 10^6$
(4) Croaker	10 ⁻⁵	10 ⁵	128		$\frac{128 \times 10^5}{1} = 1.3 \times 10^7$
(Oba market)	10 ⁻⁵	10 ⁵	65		$\frac{65 \times 10^5}{1} = 6.5 \times 10^6$
	10 ⁻⁵	10 ⁵	96	96.3 ± 18.2	$\frac{96 \times 10^5}{1} = 9.6 \times 10^6$

Table 5: Total Heterotrophic Fungi Count in Colony Forming Units per Gram of Fish Samples (For Dry Season).

Fish samples	Dilutions in Triplicate	Dilution factor	Number of colony farming unit/gm cfu/gm x 10 ³	Average number of cfu/plate	Organism/gm of fish sample
(1) Bonga	10 ⁻⁵	10 ⁵	24	$\frac{24 \times 10^3}{1}$	= 2.4 × 10 ⁶
(Koko market)	10 ⁻⁵	10 ⁵	17	20.0 ± 2.1 $\frac{17 \times 10^3}{1}$	= 1.7 × 10 ⁶
	10 ⁻⁵	10 ⁵	19	$\frac{19 \times 10^3}{1}$	1.9 × 10 ⁶
(2) Bonga	10 ⁻⁵	10 ⁵	31	$\frac{31 \times 10^3}{1}$	= 3.1 × 10 ⁶
(Oba market)	10 ⁻⁵	10 ⁵	24	28.0 ± 2.2 $\frac{24 \times 10^3}{1}$	= 5.0 × 10 ⁶

Table 5: Continue.

	10^{-5}	10^5	30		$\frac{30 \times 10^5}{1}$	2.1×10^6
(3) Croaker	10^{-5}	10^5	34		$\frac{34 \times 10^5}{1}$	$= 3.4 \times 10^6$
(Koko market)	10^{-5}	10^5	22		$\frac{22 \times 10^5}{1}$	$= 2.2 \times 10^6$
	10^{-5}	10^5	27	27.7 ± 3.5	$\frac{27 \times 10^5}{1}$	2.7×10^6
(4) Croaker	10^{-5}	10^5	25	$25 .0 \pm 1.7$	$\frac{25 \times 10^5}{1}$	$= 2.5 \times 10^6$
(Oba market)	10^{-5}	10^5	22		$\frac{22 \times 10^5}{1}$	$= 2.2 \times 10^6$
	10^{-5}	10^5	28		$\frac{28 \times 10^5}{1}$	2.8×10^6

Table 6: Frequency of Occurrence of Microbial Isolates from Fish Samples Obtained from Both Markets (For Dry Season).

Microbial Isolates	Number (%)	Fish samples			
		1	2	3	4
FUNGI					
Aspergillus niger	4 (13.3)	✓	✓	✓	✓
Mucor sp.	4 (13.3)	✓	✓	✓	✓
Saccharomyces sp.	4 (13.3)	✓	✓	✓	✓
Rhizopus sp.	1 (3.3)	✓	X	X	X
Penicillium sp	2 (6.7)	X	✓	✓	X
Neurospora sp	2 (6.7)	X	X	✓	✓
Cercaspora sp	1 (3.3)	X	X	X	✓
Bacteria					
Proteus sp	3 (10.0)	✓	✓	X	✓
Bacillus sp	2 (6.7)	✓	X	X	✓
Micrococcus sp	2 (6.7)	✓	X	✓	X
Staphylococcus aureus	3 (10.0)	X	✓	✓	✓
Serratia sp	1 (3.3)	X	✓	X	X
Enterococcus sp	1 (3.3)	X	✓	x	X
Total	30	7	7	8	8
Over all total	30 (99.9)	30 (30.0)			

Table 7: Mean Count in Colony Forming Unit per Gram of Bacteria and Fungi Isolated from Fish Samples Obtained from Koko and Oba Markets at Different Seasons. Isolates/seasons/mean Count (Cfu/gm)

Fish Samples/Locations	Dry Season	Rainy Season	Dry Season	Rainy Season
	Bacteria	Bacteria	Fungi	Fungi
Bonga/Koko	1.3×10^7	1.2×10^7	2.0×10^6	1.6×10^6
Bonga/Benin	6.4×10^6	9.7×10^6	2.8×10^6	2.0×10^6
Croaker/Koko	7.6×10^6	1.4×10^7	2.8×10^6	2.7×10^6
Croaker/Benin	9.7×10^6	1.4×10^7	2.5×10^6	3.7×10^6
$\bar{X} \pm SX$	9.2×10^6	1.2×10^7	2.5×10^6	2.5×10^6
$\bar{X} \pm SX$	1.1×10^7		2.5×10^6	

Conclusion:

This study revealed that smoke-dried fishes sold in Koko and Oba markets in both rainy and dry seasons are highly contaminated with microorganisms. Caution should be exercised in consuming smoke-dried 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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