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Microflora of fresh and smoke-dried fish in Yenagoa metropolis, Nigeria

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The microbial load of fresh and smoke-dried fish marketed in Yenagoa metropolis was evaluated. The samples were three fish types: *Clarias angularis, Channa obscura* and *Chrysicthtys auratus* from Tombia and Swali markets were analysed. The bacterial counts for fresh fish samples ranged from 4.0 x 10^8 - 2.30 x 10^{10} cfu/g while fungal counts ranged from 1.8 x 10^4 - 7.0 x 10^4 cfu/g. Bacterial and fungal counts for smoked fish were lower, ranging from 1.8 x 10^4 - 2.5 x 10^8 cfu/g and 1.0 x 10^4 - 4.0 x 10^5 cfu/g respectively. Twelve (12) bacterial isolates were obtained from fresh fish samples and 13 isolates from smoked fish. The isolated bacteria belonged to the genera *Bacillus, Pseudomonas, Proteus, Staphylococcus, Streptococcus, Corynebacterium, Lactobacillus* and *Klebsiella*. *Bacillus* was the most predominant with a frequency of occurrence of 50and 58.8% for fresh fish and smoked fish respectively. The fungi isolates belonged to *Aspergillus, Rhizopus, Penicillium*, *Saccharomyces* and *Fusarium* species. The most predominant fungi were *Aspergillus* spp and *Penicillium* spp which constituted 23.0 and 15.4% for fresh fish and smoked fish respectively.

Key words: Fish, Microflora, smoke-dried fish.

INTRODUCTION

Seafoods are vital source of food in the Niger Delta. Main seafoods consumed in the region include finfish and shrimps which are important sources of protein. Fish constitutes over 40% of the animal protein consumed by an average Nigerian (Adebayo-Tayo et al., 2008) compared to meat and it is relatively less expensive. This accounts for the mass preference for fish products. It has been reported that fishing is the major occupation in the Niger Delta (Alagoa, 1999).

Sea foods may harbour myriad microorganisms; the most prevalent being bacteria and fungi. As a result of pollution of water bodies, pathogenic organisms may be introduced to these aquatic ecosystems from which fish are harvested. Sources of pollution vary and could include faecal contamination too. As a result, water bodies may contain high numbers of coliform and these organisms would also be present in seafood harvested from such water systems.

The nutrients present in fish provide a good medium for microbial growth. Various methods have been developed to preserve fish. These include refrigeration, canning, drying and smoking (Ayers et al., 1980). The techniques employed depend on the technological advancement of the people (Adebayo-Tayo et al., 2008).In Bayelsa State, smoking is widely used for fish preservation. Smoke–drying is achieved using kilns with firewood as source of

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heat.

After processing, the products are placed in locally made baskets or jute sacs ready for transportation to various markets in the country. Often, the products are not properly packaged and stored. Consequently, re-absorption of moisture and post processing contamination of fish occur. Sikoki and Aminigo (2002) reported that changes in the moisture content of smoked fish were most significant during the first week of storage and that bacterial population increased during this period.

The aim of this study was to determine the microflora of smoke-dried fish marketed in Yenagoa, Bayelsa State.

MATERIALS AND METHODS

Source of fish samples

Fresh and smoke-dried fishes were purchased from Swali and Tombia markets in Bayelsa State. Fish belonging to three genera that is *Clarias angularis, Channa obscura,* and *Chrysicthtys auratus* were collected in sterile polythene bags and transported to the laboratory in ice.

Enumeration of bacterial/ and fungal colonies

Ten grams (10 g) of fish (only flesh and skin was used) was homogenized with 90 ml sterile distilled water for 3 min using a Kenwood blender.

Each fish sample was a composite sample obtained from 3 fishes of the same type. Tenfold serial dilution was prepared in 1% (W/V) peptone and pour plated in nutrient agar (oxoid). The nutrient agar plates were incubated at 37°C for 24 h and the colonies were counted. For the enumeration of fungal colonies, nutrient agar was replaced with malt extract agar (oxoid) in the above mentioned procedure and plates were incubated at 28±1°C for 3 - 5 days and the fungal colonies counted.

Identification of bacterial and fungal isolates

Identification of the isolated bacteria was based on cultural characteristics, cell morphology and biochemical tests (Holt et al., 1994). Fungal isolates were stained with lactophenol cotton blue and examined microscopically. The isolates were identified based on cultural characteristics, morphology of hyphae, cells and spores and kind of fruiting bodies (Barnet and Barry, 1972).

Enumeration of coliform bacteria

Coliform count was determined using the method for Most Probable Number (MPN) technique (Evans et al., 2001). The tube containing inoculated lactose broth was incubated at 35°c for 24 h and examined for gas production. For faecal coliforms, the inoculated tube of lactose broth was incubated at 44.5°C for 24 h and examined for gas production.

Statistical analysis

The data was subjected to test of difference of means using Anova with the aid of SPSS statistical software, to determine the F statistic and probability at 5% significant level (SPSS, 2010, Version 19).

RESULTS AND DISCUSSION

Microbial load of fresh and smoke-dried fish

The bacterial counts for fresh fish samples ranged from 4.0×10^8 - 2.3×10^{10} cfu/g (Table 1), while fungal counts ranged from 3.0×10^4 - 7.0×10^4 cfu/g (Table 1). The range for bacterial counts was wider than that of fungal counts. Kumolu-Johnson et al. (2010) observed an increase in microbial load of smoked C*. gariepinus* from the first day to the 28th day.

Total coliform was highest in fresh *C. angularis* from Tombia market. The smoked fish samples generally had lower bacterial counts (Table 3) than the fresh samples but fungal counts were similar for fresh and smoked fish sample. The total coliform counts for the samples ranged from 41 - 1100 MPN. Faecal coliforms were detected only in fresh fish samples. Ali et al. (2011) reported the presence of *E. coli*, *S. aureus* and fecal *streptococci* in worrying concentrations. Bouriga et al. (2012) reported that mesophiles and total coliforms increase within smoking treatment with higher levels in the traditional smoking process.

Microflora of fresh and smoke – dried fish

Twelve (12) bacterial isolates were obtained from raw fish samples (Table 2). All the isolates were rods with the exception of one. All of the isolates were catalase positive and indole negative. The bacteria belonged to five genera identified as: *B. subtilis, Corynebacterium, Lactobacillus, Pseudomonas* and *S. aureus*. Bacteria belonging to these genera have been isolated from fresh fish (Frazier and Westhoff, 1995). Most of these bacteria were also isolated from Periwinkle, a type of seafood consumed in the Niger Delta (Ndifon et al., 1999). *Bacillus* was the most predominant bacteria isolate with a frequency of 50% followed by *Pseudomonas* and *Corynebacterium* 16.6% (Figure 1a).

Thirteen (13) bacterial isolates were obtained from smoked fish samples. Eleven (11) of these were rods while two were cocci. They were all catalase positive with the exception of one isolate. The isolates belonged to six genera, namely *Bacillus, Klebsiella, Staphylococcus, Pseudomonas, Streptococcus* and *Proteus; Bacillus* had the highest frequency of occurrence (58.4%) followed by *Staphylococcus* (15.4%) while *Kelebsiella, Pseudomonas, Streptococcus* and *Proteus* had a frequency of 7.7% (Figure 1b). Aminigo and Okoro (2002) also reported the predominance of *Bacillus* and *Staphylococcus* species

Table 1. Bacterial and fungal counts for fresh and smoke-dried fish retailed in markets in Yenagoa.

Fish species	Market	Total viable count (cfu/g)	Total fungi (cfu/g)	Total coliform (MPN)	Faecal coliform (MPN)
Fresh fish					
Clarias angularis	Tombia	2.3×10^{10}	6.0×10^{4}	1100	39
Channa obscura	Tombia	2.4×10^{9}	5.0×10^{4}	460	15
Chrysicthtys auratus	Tombia	2.5×10^{9}	4.0×10^{4}	460	15
Clarias angularis	Swali	4.0×10^{8}	7.0×10^{4}	64	43
Channa obscura	Swali	4.0×10^{8}	4.0×10^{4}	75	15
Chrysicthtys auratus	Swali	6.0×10^{8}	3.0×10^{4}	120	23
Smoked fish					
Clarias angularis	Tombia	2.51×10^{8}	4.0×10^{5}	210	ND
Channa obscura	Tombia	4.4×10^{7}	1.0×10^{4}	40	ND
Chrysicthtys auratus	Tombia	5.9×10^{5}	3.0×10^{4}	23	ND
Clarias angularis	Swali	1.8×10^{4}	7.0×10^{4}	39	ND
Channa obscura	Swali	8.1×10^{7}	8.0×10^{4}	43	ND
Chrysicthtys auratus	Swali	3.1×10^{5}	5.0×10^{4}	41	ND

Table 2. Bacteria isolated from fresh fish retailed in Yenagoa.

Fish species	Cell Morphology	Gram reaction	Catalase	Oxidase	Citrate	Indole	MR	VP	H_2S	Starch Hydrolysis	Spore test	Motility	Sugar fermentation				Probable genera
													Glucose	Sucrose	Manitol	Lactose	
Clarias SP	Rod	+	+	-	+	-	+	+	-	+	+	+	A	_	A	A	*Bacillus,* SP
Clarias SP	Rod	+	+	-	+	-	+	+	-	+	+	+	A	_	A	A	*Bacillus,* SP
Channa SP	Rod	+	+	-	_	-	_	+	-	_	_	_	A/G	A	A	_	*Corynebacterium* SP
Channa SP	Rod	+	+	-	_	-	_	_	-	_	_	_	A/G	_	_	_	*Lactobacillus*
Chrysicthtys	Rod	+	+	-	+	-	+	+	-	+	+	+	A	A	A	A	*Bacillus,* SP
Chrysicthtys	Rod	+	+	-	_	-	_	+	-	_	_	_	A/G	A	A	_	*Corynebacterium* SP
Chrysicthtys	Rod	+	+	-	+	-	+	+	-	+	+	+	A	A	A	A	*Bacillus,* SP
Chrysicthtys	Rod	-	+	+	+	-	_	_	-	_	_	+	A/G	A	A	A	*Pseudomonas*
Clarias	Cocci	+	+	_	+	-	_	_	-	_	_	_	A	_	_	A	*Staphylococcus*
Clarias	Rod	+	+	_	+	-	+	+	-	+	+	+	A	A	A	A	*Bacillus,* SP
Channa	Rod	-	+	+	+	-	_	_	-	+	_	+	A/G	A	A	A	*Pseudomonas*
Channa	Rod	+	+	_	+	-	+	+	-	+	+	+	A	A	A	A	*Bacillus,* SP

in smoked fish with frequencies of occurrence of 22.2 and 18.5% respectively.

Thirteen (13) fungal isolates were obtained from fresh fish samples (Table 4) and they belonged to seven genera. The isolates were identified as *Penicillium, Candida, Saccharomyces, Aspergillus niger, Rhizopus, Mucor* and *Fusarium*. Essien et al. (2005) isolated *Fusaruim, Aspergillus, Penicillium* as toxigenic moulds from smoked shark fish. *Aspergillus* and *Penicilium* were the most predominant fungi with frequencies of 23.0and 15.4% respectively. The fungal isolates from smoke-dried samples were less varied. These were *Aspergillus, Fusarium* and *Penicillium* (Table 5). *Aspergillus species* and *Fusarium species* each had a frequency of occurrence of 42.8% thereby comprising 85.6% of fungal isolates. Abolagba et al. (2011) found that microbial contamination or recontamination of smoked catfish products varied from one locality (market) to another and even within the same locality from one fish

Table 3. Bacterial isolates from smoke dried samples.

Fish specie	Cell morphology	Gram reaction	Catalase	Oxidase	Citrate	Indole	MR	VP	H_2S	Starch Hydrolysis	Spore test	Motility	Glucose	Sucrose	Manitol	Lactose	Probable genera
Chrysicthtys	Rod	-	+	-	+	-	+	-	-	+	+	+	A/9	A/G	A/G	G	*Klebsiella* SP
Chrysicthtys	Rod	+	+	-	+	-	-	-	-	-	-	-	A	A	-	A	*Staphylococcus* SP
Channa	Rod	-	+	+	+	-	-	-	-	-	-	+	A/9	A	A	A	*Pseudomonas* SP
Channa	Rod	+	+	-	+	-	+	+	-	+	+	+	A	-	A	A	*Bacillus*
Clarias	Rod	+	+	-	+	-	+	+	-	+	+	+	A	-	A	A	*Bacillus*
Clarias	Cocci	+	-	-	+	-	-	-	-	-	-	_	A	-	-	A	*Streptococcus*
Chrysicthtys	Rod	+	+	_	+	-	+	+	_	+	+	+	A	-	A	A	*Bacillus*
Clarias	Rod	-	+	-	+	-	-	-	-	+	-	+	A	-	A	A	*Proteus*
Clarias	Rod	+	+	-	+	-	+	+	-	+	+	+	A	-	A	A	*Bacillus*
Channa	Rod	+	+	-	+	-	+	+	-	+	+	+	A	-	A	A	*Bacillus*
Channa	Cocci	+	+	-	+	-	+	-	-	-	-	-	A	A	-	A	*Staphylococcus*
Chrysicthtys	Rod	+	+	-	+	-	-	+	-	+	+	+	A	-	A	A	*Bacillus*
Chrysicthtys	Rod	+	+	-	+	-	+	+	-	+	+	+	A	-	A	A	*Bacillus*

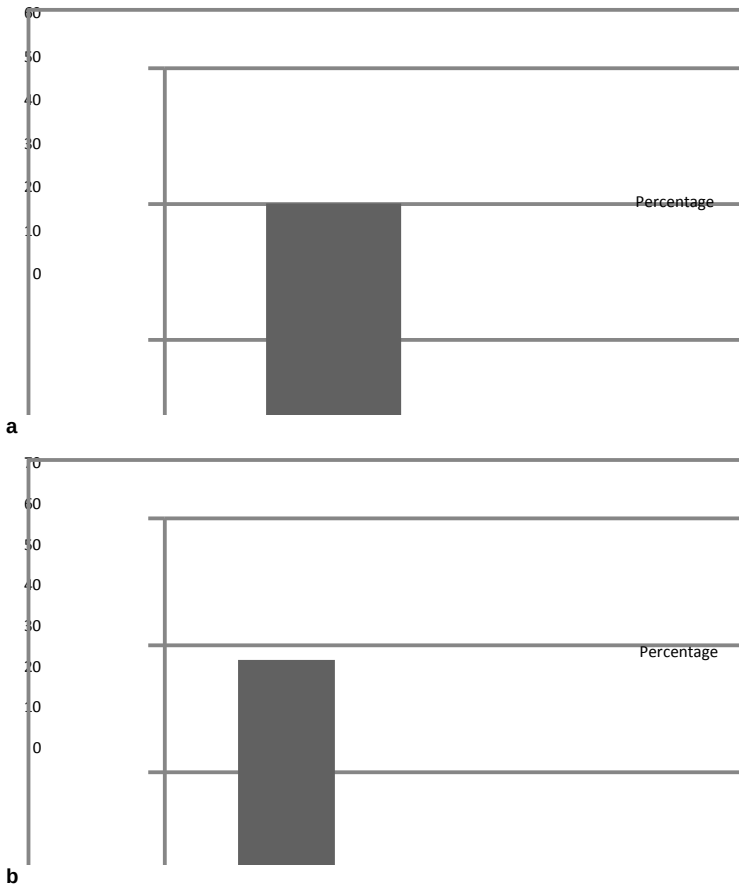

Figure 1. Frequency of occurrence of Bacteria Species in: (a). Fresh fish samples and (b). Smoked fish samples

Table 4. Characteristics of predominant fungi isolated from fresh fish samples.

Fish species fresh fish	Colony morphology	Microscopic appearance	Identity
Clarias SP	Yellowish green mycelium	Dense conidiophores, cells are branched conidiophores is smooth or rough walled, conidia is in long chains	*Penicillium* SP
Clarias SP	Smooth off white or yellow colour	Yeast like cells. Posses pseudo mycelium or true mycelium.	*Candida* SP
Channa SP	Smooth moist colonies white to cream colour	Yeast like cells only. No hyphae or pseudo hyphae.	*Saccharomyces* SP
Chrysicthtys SP	Greenish yellow mycelium	Conidiophores aseptate, unbranched with swollen apex. Conidiophores bear vesicles that produce chains of conidia.	*Aspergillus SP*
Chrysicthtys SP	Dark brown mycelium	Rhizoids brownish and unbranched the sporangiophore arising directly of up to five with smooth walls.	*Rhizopus* SP
Clarias SP	Blackish brown and yellowish brown reverse	Non- septate hyphae, conidiophores and head is radiated.	*Aspergillus* SP
Clarias SP	Smooth off white cream or yellow coloured.	Yeast like cells, posses pseudo mycelium or true mycelium.	*Candida SP*
Channa SP	Wooly white surface	Branched smooth conidiophores, brush like conidial head.	*Penicillium* SP
Channa SP	Whitish grey mycelium	Sporangiophore branched with spored sporangium, columella well developed. Rhizoids are not presents	*Mucor* SP
Channa SP	Smooth moist colonies white to cream coloured	Yeast like cells. No hyphae and pseudophyhae.	*Saccharomyces* SP
Chrysicthtys SP	Greenish yellow mycelium	Conidiophores aseptate, unbranched with swollen apex. Conidiophores bear vesicles that produce chains of conidia.	*Aspergilus* SP
Chrysicthtys SP	Yellowish green mycelium	Dense felt of conidiophores cells are branched, conidio-phores is smooth or rough walled, conidia is in long chains	*Penicillium* SP
Chrysicthtys SP	Whitish	Branched conidiophores conidia smooth or rough walled in chains or pairs	*Fusarium* SP

Table 5. Characteristics of predominant fungi isolated from smoked fish samples.

Fish species fresh fish	Colony morphology	Microscopic appearance	Identity
Clarias species	Greenish yellow mycelium	Conidiophores aseptate, unbranched with swollen apex. Conidiophores bear vesicles that produce chains of conidia	*Aspergilus* SP
Channa species	Small colony, white with greenish centre	Branched hypae with conidiophores non – septate	*Aspergilus* SP
	Fluffy spread creamy white	Macro conidia, multi cellular and sprindle shaped	*Fusarium* SP
Claria species	Whitish felty colony	Branched conidiophores. Cells are branched, conidiophores smooth or rough walled conidia is long chains.	*Fusarium* SP
Clarias species	Blackish brown and yellowish brown reverse	Non – septate hyphae, conidiophores and head radiated.	*Aspergilus* SP
Channa species	Cottony white branched like petal of flower about 9mm in diameter	Brush like conidial head smooth conidiophores phialides flask shaped.	*Penicillium* SP
Chrysicthtys species	Whitish felty colony.	Branched conidiophores. Cells are branched conidiophores is smooth or rough walled conidia is in long chains.	*Fusarium* SP

processor or seller to another.

There was no significant difference in total viable count between one market and another at 5% level of significance ($P_{70.05}$). Also, there was no significant difference in total viable count between fresh and dry fish. However, there was significant difference in faecal coliform (MPN) between fresh and dry fish at 5% significant level.

The findings of the study show that bacterial counts were generally lower for smoked fish compared to fresh fish but fungal counts were similar to both types of samples. On the other hand, bacterial genera present in fresh and smoked fish were less varied than those in fresh fish were. It is important to set microbiological standards for smoked fish products and package such products to ensure longer shelf life.

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