Microbiological and Physicochemical Level of Fresh Catfish (*Arius hendelotic)* From Different Markets in Akwa Ibom State, Nigeria

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ABSTRACT: The microbiological and physicochemical analyses of fresh catfish from different markets in Uyo, Akwa Ibom State, were carried out using standard techniques. The physiochemical component of the catfish reveals that the fish sample serve as a good source of protein and minerals element. The bacterial counts ranged from, 1.0 x 10^4 -1.1x10^6 cfu/g while the fungal counts ranged from 5.0 x 10^5-12.0 x 10^6 cfu/g. It showed that of bacterial isolated from the Catfish, *E. coli* was the most predominant organism (23.2%). This was followed by *Bacillus* spp. (15.9%), *Shigella* spp. (13.0%), *Staphylococcus* spp. (13.0%), *Micrococcus* spp. (10.2%), *Pseudomonas spp.* (8.7%) and *Enterococcus* spp. (8.7%). *Salmonella spp.* was least prevalent (7.3%). It also showed that the catfish samples from different markets harboured different level of microorganisms. Majority (40.6%) of the bacterial isolated were obtained from fishes purchased from Akpan Andem market while 22(31.9%) of the bacterial isolates were from catfishes sold at the Itu market. Fishes from the Uyo market had the least bacterial contaminations (27.5%). Also, most of the bacterial isolates were isolated from skin (34.8%), gills (33.3%) and intestines (31.9%) of the catfishes. *Mucor* spp. and *Rhizopus* spp. (37.5%) were the most predominant fungal isolates, followed by *Penicillum* spp. (18.8%). *Aspergillus spp.* was the least prevalent (6.3%). Fishes purchased from Itu market had the highest number (50.0%) of the fungal isolates and Akpan Andem market had 31.3%. Fishes from the Uyo market also had the least fungal contaminations (12.5%). *Aspergillus sp*. was only isolated from skin of the fishes from Akpan Andem market. *Penicillum* spp. was not isolated from fishes sold at Uyo market and the skin of the fishes from other location. The findings of this study showed that catfish obtained from these various markets contain potentially pathogenic microorganisms. Therefore, catfish should be properly washed and cooked before consumption.
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1. INTRODUCTION

Fish has become an increasingly important source of protein and other element necessary for the maintenance of and healthy body. Many species of fish (catfish) normally live in fresh water lakes and rivers. The term or word "Fish" are generally defined as aquatic vertebrate that are typically cold blooded covered with scales and equipped with two sets of paired fins and several unpaired fins (Helfman et al, 1997) that use gills to obtain oxygen from humbler of skeletal called Fin-Rays, put together and thus various kinds of fish greatly in shape sizes and colour (Ayres, 1995). Fish or catfish generally encompasses all sea foods including crustaceans with chitinous exoskeleton such as lobsters, crab, shrimps and such as muscle cockles and Oyster (Adam and Moses, 1995).

Fish is one of the most highly perishable food products, during handling and storage, quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product (Sallam, 2007). Marketing of Fish in Nigeria is mostly carried out by local fish sellers at ambient temperature, therefore knowledge of spoilage patterns of tropical fishes and their shelf life under ambient conditions is very important (Okoro et al., 2010). Refrigeration temperatures are also relevant because they are used by most households in Nigeria for temporary storage of fish (Okoro et al., 2010). Frozen state condition is also important since most fishes consumed in Nigeria are imported and they usually come in frozen state (Okoro et al., 2010). The quality of fish degrades, due to a complex process in which physical, chemical and microbiological forms of deterioration are implicated (Sallam, 2007).

Spoilage bacteria differ somewhat for freshwater and marine fish and for temperate and tropical water fish. Storage and processing conditions also affect microbial growth (Doyle, 2007). *Pseudomonas* and *Shewanella* are the predominant species on chilled fresh fish under aerobic conditions (Fitzgerald et al., 2003; Hozbor et al., 2006; Doyle, 2007). Packing under carbon

dioxide and addition of low concentrations of sodium chloride favour growth of lactic acid bacteria and *Photobacterium phosphoreum* (Doyle, 2007). Heavily wet-salted fish support growth of yeasts while dried and salted fish are spoiled by moulds (Doyle, 2007). Addition of organic acids selects for lactic acid bacteria and yeasts (Lyhs et al., 2004; Doyle, 2007). Pasteurization kills vegetative bacteria but spores of *Clostridium* and *Bacillus* survive and may grow, particularly in unsalted fish (Gram and Dalgaard, 2002; Doyle, 2007).

Varieties of quality attributes have been used to assess fish freshness in many cold water fish species as sea bream, sea bass, sardine and European eel (Alasalvar et al., 2001; Alasalvar et al., 2002; Ozogul et al., 2004; Ozogul et al., 2006; Hernandez et al., 2009; Liu et al., 2010). Many indices have been used for the assessment of fish quality during storage (Sallam, 2007). Such indices comprise changes in the microbial population, (Hozbor et al., 2006; Sallam, 2007), chemical changes (Dalgaard, 2000; Sallam, 2007), as well as changes in sensory attributes (Dalgaard, 2000; Sallam, 2007). However, few researches were reported on quality assessment for tropical freshwater fish species (Chytiri et al., 2004; Liu et al., 2010).

The effect of physical condition and chemical agents on the growth of microorganism in fish has been investigated and well documented and not much of information on the spoilage of fish. Some reports on the storage quality of frozen/chilled tilapia were still not comprehensive on spoilage mechanism and quality assessment (Eves et al., 1995; Al-Kahtani et al., 1996; Korel et al., 2001; Arannilewa et al., 2005; Yanar et al., 2006; Sil et al., 2008; Liu et al., 2010). In the recent time, modern biotechnology have introduced new techniques that can detect early fish contamination, improve the taste, modify the quality of fish and prolong the shelf life and also impact disease resistance to the fish (William and Michael, 2009; Okoro et al., 2010). The aim of this study is to isolate, characterize and identify the microorganisms associated with fresh fish (cat-fish) and to study the proximate mineral composition of cat fish from different market in Akwa Ibom State.

2. MATERIALS AND METHODS

2.1. Collection and processing of samples

The samples of fresh fish were collected from Itu and retail market in Uyo into sterile container and transported immediately to the laboratory for microbial and physicochemical analysis. Catfish samples were aseptically removed from the container. Using a sterile knife, the different fishes were dissected to remove the intestine, gills, skin and also the fins respectively. Each sample was blended separately for homogeneity and about 10 g was blank and shaken to mix properly.

2.2. Physico-chemical analysis

Ten grams of each catfish sample were blended with 20 ml distilled water in a blender for 30 s and pH value of fish homogenate was measured by a digital pH-meter (Meltler Delton 340 pH meter) standardized at pH 4 and 7. Samples were analyzed chemically to determine the proximate composition and mineral/heavy metals according to the method of the Association of Official Analytical Chemist (AOAC, 1990).

2.3. Microbiological examination:

The bacterial counts on the external surfaces, intestines and tissue were estimated as follows;

2.3.1. Skin surface:

A sterilised rectangular wire swab guide measuring 5cm by 2cm was placed on the lateral surface of the fish sample. A sterile cotton wool swab was dipped in 0.10% sterile peptone water and was robbed over the surface of the fish on the area covered by the wire swab guide. The swab was immediately placed in a sterile sample bottle containing 100mls of 0.10% (w/v) peptone water. The bottle was vigorously shaken for 10mins and allowed to stand for 20mins. 10 fold serial dilution of the bacterial suspension in peptone water was prepared in duplicate and viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37^0C for 48 hrs as described by Slaby *et al*. (1981).

2.3.2. Intestines, Gills and Tissues:

Ten grams of the fish sample was dissected out, blended and mixed properly. It was ascetically transferred to a sample bottle containing 90mls of 0.1% sterile peptone water. The bottle was closed and shaken thoroughly for 10 mins. and allowed to stand for 20mins, after which a 10 fold serial dilution was carried out in duplicates and viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37^OC for 48 hrs as described by Slaby *et al*. (1981).

2.4. Enumeration, isolation and identification of isolates

A ten fold serial dilution was made and appropriate dilutions were used for microbial enumeration using standard pour plate method. The pour plates were made using Nutrient agar (NA), MacConkey agar (MCA), Salmonella/Shigella agar (SSA), Thiosulphate citrate bile salt sucrose sugar agar (TCBS) and Sabouraud Dextrose agar (SDA) for total heterotrophic bacterial counts, total

coliform counts, total Salmonella/Shigella counts, total vibrio count and total fungal counts respectively. The plates were incubated at 37^{O}C for 24-48h except for fungal plates which were incubated at room temperature (28±2 ^{O}C) for 3-7 days. Discrete colonies were sub-cultured into fresh agar plates aseptically to obtain pure cultures of the isolates. Colonies identifiable as discrete were carefully examined macroscopically for cultural characteristics. All isolates were Gram stained to determine their gram reaction. The isolates were identified by comparing their characteristics with those of known taxa, as described by Jolt et al. (1994), Cheesbrough (2006) and Oyeleke and Manga (2008). Fungal isolates were sub-cultured on SDA and identified based on their morphological and cultural characteristics.

3. RESULTS ANALYSIS

3.1. Physico-chemical and nutritional (proximate) composition of Catfish samples

Table 1 showed the proximate composition of the fish samples. The compositions were ash, moisture, calcium, crude protein etc. Table 1 showed the presence of the physical, chemical and nutritional composition of the fish sample which is moisture content, ash content, crude protein, calcium, potassium etc. The pH values ranged from 7.23 to 8.88, with those from Akpan Andem market having the highest pH values (8.88). The moisture contents ranged from 375.0 to 378.0, with the fish samples from Itu market having the highest moisture content (378.0), followed by those from Akpan Andem market (377.0). Ash content ranged from 13.40 to 14.80. Fishes from Itu and Akpan Andem market (13.40) had the lower ash contents compared to those from Uyo (14.80). Crude protein values ranged from 240.27 to 247.80 and this was low in samples from Itu (240.27) and the highest from fish samples from Akpan Andem market (247.80). Crude fibre was higher in fishes from Akpan Andem markets (46.00) compared to those from Itu (24.32) and Uyo (35.60). Lipid content was highest in samples from Akpan Andem markets (78.30), followed by those from Uyo (76.20) and those from Itu had the lowest lipid content (65.00). Total carbohydrate ranged from 80.00 to 85.35. Fishes from Akpan Andem market had the highest total carbohydrate content compared to those from the other two markets. Calcium and potassium ion content were highest in fishes from Akpan Andem market. Phosphorous content was highest in fishes from Uyo market and iron content was highest in fishes from Akpan Andem market as shown in Table 1.

Table 1: Physico-chemical and nutritional composition of Catfish samples

Parameters	Itu market	Uyo market	Akpan Andem market
pH	7.23	7.35	8.88
Moisture conten	378.0	375.0	377.0
Ash content	13.40	14.80	13.40
Crude protein	240.27	241.58	247.80
Crude fibre	24.32	35.60	46.00
Lipid content	65.00	76.20	78.30
Total Carbohydrate	80.00	81.20	85.35
Calcium	1537.04	3018.52	5086.42
Potassium	620.40	750.70	870.90
Phosphorus	536.80	640.40	396.00
Iron	552.20	526.0	566.0

3.2. Total bacterial and fungal Counts

Table 2 shows the total bacterial and total fungal count of the catfish samples from different markets in Akwa-Ibom State, Nigeria. The bacterial counts ranged from, 1.0 x10^4-1.1x10^6 cfu/g while the fungal counts ranged from 5.0 x 10^4 -12.0 x 10^6 cfu/g. The bacterial load of the catfish samples were within the range of the maximum limit (10^6 cfu/g) recommended by the international commission for microbiological standards of foods (ICMSF, 1978; Okoro et al., 2010).

Table 2: Total viable count for Bacterial and fungal isolates

Locations	Total Bacterial Count (Cfu/g	Total Fungal Count (Cfu/g)
Itu	1.1 x 10^5	12.0x10^6
Uyo	1.0 x 10^4	5.0x10^5
Akpan Andem	1.1 x 10^6	7.0x10^6

3.3. Frequency of occurrence of bacterial isolates

Table 2 shows the frequency of occurrence of bacterial isolates. A total of 69 bacterial isolates were obtained from the catfish sold in Uyo, Itu and Akpan Andem markets all in Uyo metropolis. The isolates were identified as *Shigella* spp., *Enterococcus* spp., *Bacillus* spp., *Micrococcus* spp., *Salmonella* spp., *Pseudomonas* spp., *Escherichia coli*, and *Staphylococcus spp.* Table 2 showed the frequency of occurrence of bacterial isolates from Cat fish. It showed that *E. coli* was the most predominant organism (23.2%). This was followed by *Bacillus* spp. (15.9%), *Shigella spp.* (13.0%), *Staphylococcus* spp. (13.0%), *Micrococcus* spp. (10.2%), *Pseudomonas* spp. (8.7%) and *Enterococcus* spp. (8.7%). *Salmonella* spp. was least prevalent (7.3%). Table 2 showed different

microorganisms isolated from the different parts of the fish samples. It showed that the fish samples from different markets harboured different level of microorganisms. Majority (40.6%, n=28) of the bacterial isolates were obtained from fishes purchased from Akpan Andem market while 22(31.9%) of the bacterial isolates were obtained from those sold at the Itu market. Fishes from the Uyo market had the least bacterial contaminations (27.5%). Also, most of the bacterial isolates were isolated from skin (34.8%, n=24) of the fishes. This was followed by gills (33.3%, n=23) of the fishes and intestines (31.9%, n=22) had the least the bacterial contamination as shown in Table 3.

Table 2: Frequency of occurrence of bacterial isolates

Isolates	No. (%)	Fish parts (%)			Market locations (%)		
		Gills	Intestine	Skin	Itu	Uyo	Akpan Andem
Bacillus spp	11(15.9)	5(45.5)	3(27.3)	3(27.3)	4(36.4)	3(27.3)	4(36.4)
Enterococcus spp	6(8.7)	1(16.7)	3(50.0)	2(33.3)	1(16.7)	2(33.3)	3(50.0)
Escherichia coli	16(23.2)	5(31.3)	4(25.0)	7(43.8)	6(37.5)	4(25.0)	6(37.5)
Micrcoccus spp	7(10.2)	3(42.9)	3(42.9)	1(14.3)	1(14.3)	2(28.6)	4(57.1)
Pseudomonas spp	6(8.7)	1(16.7)	2(33.3)	3(50.0)	2(33.3)	1(16.7)	3(50.0)
Salmonella spp	5(7.3)	2(40.0)	2(40.0)	1(20.0)	1(20.0)	3(60.0)	1(20.0)
Shigella spp	9(13.0)	4(44.4)	2(22.2)	3(33.3)	4(44.4)	2(22.2)	3(33.3)
Staphylococcus spp	9(13.0)	2(22.2)	3(33.3)	4(44.4)	3(33.3)	2(22.2)	4(44.4)
Total	**69(100.0)**	**23(33.3)**	**22(31.9)**	**24(34.8)**	**22(31.9)**	**19(27.5)**	**28(40.6)**

3.4. Frequency of occurrence of fungal isolates

Table 3 shows the frequency of occurrence of fungal isolates. Sixteen fungal isolates were obtained in this study. The fungal isolates were identified as *Aspergillus* spp., *Mucor* spp., *Rhizopus* spp., and *Penicillum* spp. It showed that *Mucor* spp. and *Rhizopus* spp. (37.5%) were most predominant fungal isolates, followed by *Penicillum* spp. [3(18.8%). *Aspergillus* spp. was the least frequent fungal isolates with 6.3%. Eight (50.0%) of the fungal isolates were obtained from fishes purchased from Itu market. Five (31.3%) of the fungal isolates were obtained from those sold at the Akpan Andem market. Fishes from the Uyo market had the least fungal contaminations (12.5%). Also, *Aspergillus* sp. was only isolated from skin of the fish purchased from Akpan Andem market. No *Penicillum* spp. was isolated from fishes from Uyo market and the skin of the fishes from other location as shown in Table 3.

Table 3: Frequency of occurrence of fungal isolates

Fungal isolates	No. (%)	Fish parts (%)			Market locations (%)		
		Gills	Intestine	Skin	Itu	Uyo	Akpan Andem
Aspergillus sp	1 (06.3)	0(00.0)	0(00.0)	1(100.0)	0(00.0)	0(00.0)	1(100.0)
Mucor sp	6 (37.5)	2(33.3)	2(33.3)	2(33.3)	3(50.0)	1(16.7)	2(33.3)
Penicillium sp	3 (18.8)	2(66.7)	1(33.3)	0(00.0)	1(33.3)	0(00.0)	1(33.3)
Rhizopus sp	6 (37.5)	1(16.7)	3(50.0)	2(33.3)	4(66.7)	1(16.7)	1(16.7)
Total	**16(100.0)**	**5(31.3)**	**6(37.5)**	**5(31.3)**	**8(50.0)**	**2(12.5)**	**5(31.3)**

4. DISCUSSION

From the results of the microbial and physiochemical analysis of catfish sold in Uyo, Itu and Akpan Andem markets in Akwa Ibom State, it reveal that many pathogenic bacteria were obtained which are *Pseudomonas* spp, *Bacillus* spp, *Entercoccus* spp, *Micrococcus* spp, while the fungal isolates obtained were *Aspergillus* spp, *Rhizopus* spp, *Mucor* spp *and Penicillum* spp. From the number of isolates obtained from the fishes obtained in Uyo, it shows that catfish obtained from these market (Itu, Uyo and Akpan Andem) had the highest frequency of occurrence of the bacterial isolates. From the previous work on catfish, the microorganism isolated from the different parts of these catfish samples such as intestine, gills and skin showed slight differentiation. The skins of these catfishes contained more of the bacterial and fungal isolates due to their constant contact with the water.

Spore-forming bacteria are usually associated with spoilage of heat-treated foods because their spores can survive high processing

temperatures (Doyle, 2007). These Gram-positive bacteria may be strict anaerobes or facultative (capable of growth with or without oxygen) (Doyle, 2007). Other thermophiles (*Bacillus* and *Geobacillus* spp.) cause a flat sour spoilage of high or low pH canned foods with little or no gas production, and one species causes ropiness in bread held at high ambient temperatures (Pepe et al., 2003; Doyle, 2007). Mesophilic anaerobes (*Bacillus* spp.) growing at ambient temperatures, cause several types of spoilage of vegetables (Chang and Kang, 2004; Doyle, 2007). Psychrotolerant spore-formers produce gas and sickly odours in chilled meats and brine-cured hams (*Clostridium* spp.) while others produce off-odors and gas in vacuum-packed, chilled foods and milk (*Bacillus* spp.) (Doyle, 2007).

Pseudomonas and related genera are aerobic, Gram-negative soil bacteria, some of which can degrade a wide variety of unusual compounds (Doyle, 2007). They generally require a high water activity for growth (0.95 or higher) and are inhibited by pH values less than 5.4 (Doyle, 2007). Some species grow at refrigeration temperatures (psychrophilic) while other are adapted for growth at warmer, ambient temperatures (Doyle, 2007). Four species of *Pseudomonas* (*P. fluorescens, P. fragi, P. lundensis,* and *P. viridiflava*), are the main food spoilage organisms (Doyle, 2007). *P. fluorescens, P. fragi,* and *P. lundensis* cause spoilage of animal-derived foods (meat, fish, milk) by secreting lipases and proteases that cause formation of sulfides and trimethylamine (off-odours) and by forming biofilms (slime) on surfaces (Fitzgerald et al., 2003; Hozbor et al., 2006; Doyle, 2007). Some strains are adapted for growth at cold temperatures and spoil these foods in the refrigerator (Doyle, 2007). *Pseudomonas* spp. was reported as one of the dominant bacteria during the study period. *Pseudomonas* spp. was reported as the dominant bacteria during the ice stored period in a study by Hozbor et al. (2006).

The presence of *Aspergillus* spp reported in this study brings to mind the likely presence of toxin produced by this fungal isolate. This could become the possible source Aspergillosis transmission among consumer. *Aspergillus* and related molds generally grow faster and are more resistant to high temperatures and low water activity than *Penicillium* spp. and tend to dominate spoilage in warmer climates (Doyle, 2007). Many aspergilli produce mycotoxins: aflatoxins, ochratoxin, territrems, cyclopiazonic acid (Doyle, 2007). Aspergilli spoil a wide variety of food and non-food items (paper, leather, etc.) but are probably best known for spoilage of grains, dried beans, peanuts, tree nuts, and some spices (Doyle, 2007). *Penicillium* and related genera are present in soils and plant debris from both tropical and Antarctic conditions but tend to dominate spoilage in temperate regions (Doyle, 2007). They are distinguished by their reproductive structures that produce chains of conidia. Although they can be useful to humans in producing antibiotics and blue cheese, many species are important spoilage organisms, and some produce potent mycotoxins (patulin, ochratoxin, citreoviridin, penitrem) (Doyle, 2007

The physicochemical component of the catfish reveals that the fish sample serve as a good source of protein and minerals element. However, the presence of some pathogenic bacterial isolates such as *Escherichia coli* reveals the pollution of the aquatic habitats with faecal matter either from sewage disposal or from human activities. It can also be observed that the fishes sold in Itu Market contains high microbial load compared to other fishes which may be due to human activities that are carried out there such as bathing, washing, defecating etc. Most of the organisms isolated causes food poisoning such as shigellosis, Salmonellosis caused by *Shigella* and *Salmonella* spp respectively. *Bacillus* which is known to be the highest occurring bacterial isolate causes toxin-medicated disease rather than an infection. This study revealed that the fish samples from Itu Market have high microbial loads of pathogenic microorganisms. The high microbial load reported in this study may have been due to high level of contaminants in Itu River where these catfish were obtained. However, there should be proper processing, storage and handling measure. People should be oriented not to buy fishes that have shown signs of spoilage.

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