Microbiological and physiochemical changes and its correlation with quality indices of tilapia fish (*Oreochromis niloticus*) sold in Itu and Uyo markets in Akwa Ibom State, Nigeria

[1]Adebayo-Tayo BC, [2]Odu NN, [2]Okonko IO

[1]Department of Microbiology, University of Ibadan, Ibadan, Nigeria
[2]Department of Microbiology, University of Port Harcourt, Choba, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria;
mac2finney@yahoo.com;iheanyi.okonko@uniport.edu.ng; Tel.: +2348035380891

Abstract : *Shigella sp*, *Micrococcus sp*, *Salmonella sp* and *Staphylococcus sp* was the dominant bacteria during the microbial, physiochemical and proximate analysis of tilapia fishes sold at Itu and Uyo market in Akwa Ibom State, Nigeria. In tilapia fish samples from Itu market, moisture content was highest with it ranging from (40.0-37.7) and lower in Uyo with (17.4-37.8). Crude protein was low in samples from Itu with (34.30-35.0) and highest in Uyo with (34.78-40.0); ash content for Itu is (43.40-44.80) and (43.40-59.30) for Uyo. Crude fibre was higher in Uyo with (44.50-55.33) and Itu with (35.33-40.33) while lipid content was highest in samples from Itu with (76.20-79.00) and Uyo with (69.70-75.10). It also showed the total heterotrophic count, total coliform count and total fungal count of the isolates obtained from fish samples from Itu market was greater than that from Uyo market. Samples from Uyo market have the total heterotrophic count which ranged from 9.0×10^7 cfu/g to 1.10×10^9 cfu/g, total coliform count 5.0×10^7 cfu/g to 1.1×10^8 cfu/g while the total fungal count from 7.0×10^6 cfu/g to 1.2×10^7 cfu/g. The total heterophic count of the samples from Itu market ranged from 1.5×10^8 cfu/g to 8.0×10^8 cfu/g, the total coliform count ranged from 6.5×10^7 cfu/g to 1.0×10^8 cfu/g while the total fungal count from 5.0×10^6 cfu/g to 9.0×10^6 cfu/g. Among the bacteria isolated from tilapia fish, *Shigella sp* [4(30.8%)] was the most prevalent. This was followed by *Micrococcus sp* [3(23.1%)], *Salmonella sp* [2(15.4%)], and *Staphylococcus sp* [2(15.4%)] while *Pseudomonas sp* and *Escherichia coli* was the least prevalent [1(7.7%). Of the fungal isolates identified *Mucor sp* and *Rhizopus sp* were the most predominant having 33.3% each. This was followed by *Aspergillus sp* and *Penicillium sp* (16.7%). The findings of this study showed that the samples of tilapia fish from Itu and Uyo market were contaminated with potential pathogenic microorganisms. Thus, tilapia fish should be cooked properly before consumption.

1. Introduction

The term 'Fish' encompasses all sea foods including crustaceans with a chitinous exoskeleton such as lobsters, crabs, shrimps and mollusks such as mussles cockles, clams and oysters (Adams and Moss, 1995). Fish are aquatic Vertebrate that is typically cold. There are more kinds of fishes than all other kinds of water and land Vertebrates put together and this various kind of fish differ so greatly in shape, colour and sizes (Ayres, 1993).

Fish as earlier defined are generally vertebrates that use gills to obtain oxygen from water and have fins with variable number of skeletal elements called fin rays (Zapata et al 1996). The physiochemical levels of these fishes found in Habitats that have already being polluted by industrial discharge and waste disposal may either kill these fishes or render them inedible which if not taken note of might cause a great deal of harm to man and his environment.

Fish is a very perishable, high-protein food that typically contains a high level of free amino acids. Microbes metabolize these amino acids, producing ammonia, biogenic amines such as putrescine, histamine, and cadaverine, organic acids, ketones, and sulfur compounds (Emborg et al., 2005; Olafsdottir et al., 2005; Baixas-Nogueras et al., 2005; Dalgaard et al., 2006; Doyle, 2007). Degradation of lipids in fatty fish produces rancid odors (Haugen and Undeland, 2003; Doyle, 2007). In addition, marine fish and some freshwater fish contain trimethylamine oxide that is degraded by several spoilage bacteria to trimethylamine (TMA), the compound responsible for fishy off odors. Iron is a limiting nutrient in fish, and this favors growth of bacteria such as pseudomonads that produce siderophores that bind iron (Gram and Dalgaard, 2002; Doyle, 2007).

Tilapia are ranked as the second most widely farmed fish in the world. They are farmed in at least 85 countries, with most production coming from Asia and Latin America (Eknath et al., 2007; Liu et al., 2010). In 2007, tilapia production of China reached 1,210,000 tons, approximately up to 49% of the global yield (Li and Cai, 2008; Liu et al., 2010). The majority (approximately 66.7%) of tilapia production in China is sold alive in domestic market and the remaining are frozen for exportation or used for further processing (Li and Cai, 2008; Liu et al., 2010).

Fish spoilage is a complex process in which physical, chemical and microbiological mechanisms are implicated (Hozbor et al., 2006). Some reports on the storage quality of frozen/chilled tilapia were still not comprehensive on spoilage mechanism and quality assessment (Arannilewa et al., 2005; Yanar et al., 2006; Sil et al., 2008; Liu et al., 2010).

In this study, the type and species of fish was narrowed down to the tilapia fish and the study aimed at determining the microbial level of these fish (Tilapia) from the different markets, isolating, characterizing and identifying microorganisms in the fish samples and determining its physiochemical level and proximate compositions.

2. Materials and Methods
2.1. Collection and processing of samples
The samples of fresh tilapia fish were collected from Itu and retail market in Uyo into sterile container and transported immediately to the laboratory for microbial and physiochemical analysis. Tilapia fish samples were aseptically removed from the container. Using a sterile, the different fishes were dissected to remove the intestine, gills, skin and also the fins respectively. Each sample was blended separately for homogenicity and about 10g taken for microbiological analysis.

2.2. Skin:
Samples of raw tilapia fish from different locations were collected, of which the skin of raw fish was taken by rubbing the sterilized cotton swab over the skin and then inoculating into the nutrient broth.

2.3. Gills:
The sterilized cotton swab was wiped against the gill filaments by lifting the operculum with the help of a pair forceps. The sample was inoculated in the nutrient broth as well as swabbed on nutrient agar. A part of the gill filament removed aseptically was also placed in a separate (nutrient broth, MacConkey broth and Selinite F broth tubes) in order to isolate all the bacteria present on the gill filaments which might have escaped contact with the swab. The examined gills were taken from raw tilapia fish.

2.4. Intestine and muscles:
This was done by cutting a part of intestine and muscle after sterilizing with red hot scalped and inoculation in the media (nutrient broth, MacConkey broth and Selinite F broth tubes). The samples included intestines and muscles from raw tilapia fish.

2.5. Fins:
This was done by cutting the fins of tilapia fish samples after sterilizing with red hot scalped and inoculation in the media (nutrient broth, MacConkey broth and Selinite F broth tubes). The samples included fins from raw tilapia fishes.

2.6. Physico-chemical analysis
Samples were analyzed chemically to determine the proximate composition according to the method of the Association of Official Analytical Chemist (AOAC, 1990). The proximate composition was carried out according to the method of AOAC (1990). This includes determination of pH, moisture content, ash content, crude fat, crude fiber, crude protein and carbohydrate. The pH readings were taken according to the method of AOAC (1990) using a pH meter (Meltler Delton 340 pH meter). Samples of tilapia fishes were analyzed for calcium Ca^{2+}, potassium (K^+), phosphorous (P^{2+}), iron (Fe^{2+}) according to the methods of AOAC (1990), Fishwick and Wright method of 1977, Osborne and Voogt method of 1978, Macro Kjeldahl method of Osborne and Voogt, 1978. Sample solution was prepared by homogenizing 10g of the sample in 100ml distilled water to form slurry.

2.7. Enumeration, isolation and identification 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), and Sabouraud Dextrose agar (SDA) for total heterotrophic bacterial counts, total coliform counts, and total fungal counts respectively. The plates were incubated at 37^OC 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) and Oyeleke and Manga (2008). Fungal isolates were subcultured on SDA and identified based on their morphological and cultural characteristics.

2.8. Statistical Analysis

The data obtained were subjected to analysis of variance (ANOVA) using Graph Pad Prism Software, version 5.01. Significant difference between means were determined at $p < 0.05$.

3. Results Analysis

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. In samples gotten from Itu market moisture content was highest with it ranging from (40.0-37.7) and lower in Uyo with (17.4-37.8). Crude protein was low in samples from Itu with (34.30-35.0) and highest in Uyo with (34.78-40.0); ash content for Itu is (43.40-44.80) and (43.40-59.30) for Uyo. Crude fibre was higher in Uyo with (44.50-55.33) and Itu with (35.33-40.33) while lipid content was highest in samples from Itu with (76.20-79.00) and Uyo with (69.70-75.10) as shown in Table 1.

Table 1: Physico-chemical and nutritional composition of Tilapia fish samples

	ITU MARKET		UYO MARKET	
	Sample 1	Sample 2	Sample 1	Sample 2
pH	8.88	9.00	7.23	7.35
Moisture content	37.30	40.00	37.80	37.40
Ash content	43.40	44.80	43.40	50.30
Crude protein	34.00	35.27	34.80	40.10
Crude fibre	35.32	40.33	55.33	44.50
Lipid content	79.00	78.20	69.70	75.10
Total Carbohydrate	80.00	81.20	85.35	76.10
Ca^{2+}	1540.40	3018.52	5086.42	4032.40
K^{+}	620.40	750.70	870.90	920.70
P^{2+}	536.80	640.70	570.70	610.10
Fe^{2+}	552.20	579.0	620.0	588.0

Table 2 showed the total heterotrophic count, total coliform count and total fungal count of the isolates obtained from fish samples from Itu market was greater than that from Uyo market. Samples from Uyo market have the total heterotrophic count which ranged from 9.0×10^7 cfu/g to 1.10×10^9 cfu/g, total coliform count 5.0×10^7 cfu/g to 1.1×10^8 cfu/g while the total fungal count from 7.0×10^6 cfu/g to 1.2×10^7 cfu/g. The total heterophic count of the samples from Itu market ranged from 1.5×10^8 cfu/g to 8.0×10^8 cfu/g, the total coliform count ranged from 6.5×10^7 cfu/g to 1.0×10^8 cfu/g while the total fungal count from 5.0×10^6 cfu/g to 9.0×10^6 cfu/g (Table 2).

Table 2: Total viable count for Bacterial and fungal isolates

Samples	Total Heterotrophic Count (Cfu/g)	Total Coliform Count (Cfu/g)	Total Fungal Count (Cfu/g)
Itu Market			
A	1.5×10^8	8.0×10^7	5.0×10^6
B	1.7×10^8	9.0×10^7	7.0×10^6
C	1.13×10^8	7.5×10^7	9.0×10^6
D	1.6×10^8	1.0×10^8	8.0×10^6
E	8.0×10^8	6.5×10^7	6.0×10^6
Uyo Market			
A	9.0×10^7	5.0×10^7	7.0×10^6
B	1.5×10^8	1.2×10^7	1.0×10^7
C	1.3×10^8	1.1×10^8	1.1×10^7
D	1.2×10^8	1.0×10^8	9.0×10^6
E	1.1×10^9	8.0×10^7	1.2×10^7

The 13(100.0%) bacterial isolates obtained from Tilapia fish sold in Uyo and Itu markets were identified as *Pseudomonas sp*, *Micrococcus sp*, *Salmonella sp*, *Eschericha coli*, *Bacillus sp*, *Staphylococcus sp*, and *Shigella sp*. as shown in Table 3. Table 3 showed the bacterial isolates obtained from the fish samples and their percentage of occurrence with *Shigella sp* [4(30.8%)] having the highest frequency of occurrence. This was followed by *Micrococcus sp* [3(23.1%)], *Salmonella sp* [2(15.4%)], *Staphylococcus sp* [2(15.4%)]. *Pseudomonas sp* and *Escherichia coli* was the least occurring microorganisms having [1(7.7%) each.

Table 3: Percentage frequency of occurrence of bacterial isolates

Isolates	No. (%)
Escherichia coli	1(7.7)
Micrococcus sp	3(23.1)
Pseudomonas sp	1(7.7)
Salmonella sp	2(15.4)
Shigella sp	4(30.8)
Staphylococcus sp	2(15.4)
Total	**13(100.0)**

From the result obtained, Table 4 showed the presence of 6(100.0%) fungal isolates from the fish samples which were identified as *Aspergillus sp*, *Rhizopus sp*, *Mucor sp* and *Penicillium sp*. These organisms contributed to spoilage of the fish samples. The frequency of occurrence showed that *Mucor sp* and *Rhizopus sp* were the most predominant fungal isolates having 33.3% (n=2) each. This was followed by

Aspergillus sp and *Penicillium sp* each having 16.7% (n=1).

Table 4: Percentage frequency of occurrence of fungal isolates

Fungal isolates	No. (%)
Aspergillus sp	1 (16.7)
Mucor sp	2 (33.3)
Penicillium sp	1 (16.7)
Rhizopus sp	2 (33.3)
Total	**6(100.0)**

4. DISCUSSION

From the result of the microbiological and physiochemical analysis of tilapia fish sold in Itu and Uyo market in Akwa-Ibom State, it can be deduced that many potential pathogenic bacteria were isolated from the fish samples which are *Pseudomonas sp, Micrococcus sp*, *Salmonella sp*, *Escherichia coli*, *Bacillus sp*, *Staphylococcus sp*, *Enterococcus sp* and *Shigella sp*. Also, the fugal isolated obtained from the samples also include *Aspergillus sp*, *Rhizopus sp*, *Mucor sp* and *Penicillium sp*. The microorganisms reported in this study are similar to what has been reportedly isolated in other studies in Nigeria (Okonko et al., 2008, 2009; Adebayo-Tayo et al., 2012a,b). In a study by Yagoub (2009), *Enterobacteriaceae* were isolated from gills, skin, muscles and the intestine of randomly collected fishes. Thampuran et al. (2005) reported that the microbial quality of the tilapia indicated that all tissue samples except muscle tissues were contaminated with fecal coliform where *Escherichia coli* was the most common contaminant and is often encountered in high numbers. This is also in accordance with what was previously reported by Koutsoumanis and Nychas (2000), Jeyasekaran et al. (2006), Yagoub (2009) and Adebayo-Tayo et al. (2012a,b) who identified *pseudomonads* as a good spoilage index.

In this study, samples of tilapia fish from Uyo market have the total heterotrophic count which ranged from 9.0×10^7 cfu/g to 1.10×10^9 cfu/g, total coliform count 5.0×10^7 cfu/g to 1.1×10^8 cfu/g while the total fungal count from 7.0×10^6 cfu/g to 1.2×10^7 cfu/g. The total heterophic count of the samples from Itu market ranged from 1.5×10^8 cfu/g to 8.0×10^8 cfu/g, the total coliform count ranged from 6.5×10^7 cfu/g to 1.0×10^8 cfu/g while the total fungal count from 5.0×10^6 cfu/g to 9.0×10^6 cfu/g Arannilewa et al. (2006) also found that the total coliform count range in fish was between 3.0×10^3 - 7.5×10^6 with increasing values, as the duration of storage increases. Hood et al. (1983) found that fecal coliform levels were above the recommended wholesale level suggested by the National Shellfish Sanitation Program (less than or equal to 230/100 g). This is in agreement with earlier report by Agbu et al. (1998) in Kastina in terms of high viable counts of coliform density in the water ecosystem.

The presence of coliforms from the fish samples such as *Escherichia coli, Enterococcus* sp. and *Salmonella* sp. reveals the contaminations of the habitat by faecal matters. It can also be deduced that the tilapia fish samples from Itu market contained high microbial load than the tilapia fish samples from Uyo market. *Escherichia coli* cause dysentery (Nester et al., 1995). Normal fish and human skin is a complex organ and the bacterial populations associated with it are complex in kind and number. The skin supports the growth of both aerobic and anaerobic bacteria (Nester et al., 1995).

All the organisms isolated from the samples of tilapia fish have health implication for man except *Micrococcus* sp., which have not been associated with human infections. Most of these organisms are associated with food poisoning infection and typhoid fever in humans, shigellosis food infection in humans, and the presence of *Aspergillus* reveals possible production of aflatoxins (Nester et al., 1995; Adebayo-Tayo et al., 2006). *Escherichia coli* is implicated in newborn meningitis and infantile diarrhea, *Salmonella paratyphi* is the causative agent of paratyphoid fever in humans, who are the only reservoir of this organism (Nester et al., 1995; Adebayo-Tayo et al., 2006). *Enterococcus* sp. has been implicated in human infections like pharyngities, scarlet fever and pneumonia (Adebayo-Tayo et al., 2009).

Staphylococcus aureus, a mesophile have been implicated in food poisoning outbreak of some food material (Adebayo-Tayo et al., 2009). Odunfa (1988) reported that *S. aureus* levels of 10^8 ml are considered potential hazardous to consumers. The presence of *S. aureus* is an indication of contamination by food handlers and 80% of them are being harbored by man as normal micro flora (Adebayo-Tayo et al., 2009). *Bacillus sp* causes a toxin-medicated disease rather than infection such as diarrhea and emetic illness characterized by nausea and vomiting (Bergdoll, 1981; Adebayo-Tayo et al., 2006, 2009). *Pseudomonas sp* on the other hand is prevalent among patients with wounds, burns, cystic fibrosis are likely to have introduced into the environment by swimmers and infected individuals who use these waters were the tilapia samples were obtained for recreational purposes (Adebayo – Tayo et al., 2006).

The contamination may be as a result of human activities such as deposition of faecal matters, washing, bathing, discharge of effluents into the rivers were these fish are harvested from. The occurrences of *Bacillus* sp. can be said to be as a result of prevalence of their spores in the environment (Jay, 1978; Adebayo-Tayo et al., 2009). *Bacillus* species are spore formers whose spores could survive high temperatures of processing. *Bacillus* sp. has been isolated from non-alcoholic beverages (Osuntogun and Aboaba, 2004; Amusa et al., 2005). The organisms are present in most raw materials used in food manufacturing at concentration of 10^3/g or less. The infectious dose has been estimated to be 10^5/g (Adeleke and Odeola, 1997; Adebayo-Tayo et al., 2009)

The presence of highly pathogenic bacterial isolates, like *Bacillus* sp., *Salmonella* sp., *Shigella* sp., *E. coli*, *Pseudomonas* sp. and *S. aureus* are organisms of public health concern. The presence of these microbes is an indication of possible contamination resulting from the use of well water, which is mostly used in local food processing industries are not free from microbial contamination (Potter, 1983; Adebayo-Tayo et al., 2009). *S. aureus* known for production of heat stable enterotoxin (Stewart, 1974) and potentials for multiple antibiotic resistances when they get into the living tissue (Foster, 1996; Allen and Cowan, 1997; Okuma et al., 2002; Scott, 2002; Klein et al., 2007) makes the product of immense epidemiological danger (Adebayo-Tayo et al., 2009).

Aspergillus spp. have also been implicated in causing mycetoma in human (Cheesbrough, 2000; Adebayo-Tayo et al., 2009). *A. flavus* is involved in allergic (aspergillosis (pulmonary aspergillo-sis) and also produces aflatoxin that is highly carcinogenic (Prescott et al., 2005; Adebayo-Tayo et al., 2009). The presence of species of Aspergillus could be attributed to the prevalence of their spores in the atmosphere (Adebayo-Tayo et al., 2009). This organism was easily trapped during the post harvest processing and handling of tilapia fishes. Since most fungal spores are found in the air, the spores must have contaminated the tilapia fishes during storage, transportation and displaying of the fishes at the market. The liberated spore can easily settle on food and ceilings of room and then germinate (Okhuoya and Ayanlola, 1986). Dongo and Ayodele (1997) have shown that Aspergillus occurred highest in the number of colonies identified from air spora of some localities. Ilodu and IIoh (2007) isolated and identified *A. flavus* and *A. niger* from sorrel drink.

Penicillium spp. cause visible rots on citrus, pear, and apple fruits and cause enormous losses in these crops (Doyle, 2007). They also spoil other fruits and vegetables, including cereals. Some species can attack refrigerated and processed foods such as jams and margarine (Doyle, 2007). A related genus, *Byssochlamys*, is the most important organism causing spoilage of pasteurized juices because of the high heat resistance of its spores (Doyle, 2007).

The physiochemical and Nutritional composition of the tilapia fish samples revealed that they serve as food sources of protein and mineral elements (Briggs and Colloway, 1999). From the physiochemical analysis, samples from Itu had the highest moisture content than does from Uyo, these suggest that these fish samples analyzed may not be storable for a long while due to the high water activity. Crude protein was high in Uyo samples Itu which is probably due to favourable environmental conditions. The high level of protein source for the alleviation of Kwashiorkor arising from proteins malnutrition especially in diets meant for children. The high ash contents of the samples from Uyo compared to Itu may be due to pollution of these aquatic habitats were the fish samples were gotten by crude oil. This also suggests higher level of mineral elements in these samples as against non contaminated areas, since uptake of water, salts and mineral elements are carried out by these fish samples. These samples irrespective of source (i.e. Itu and Uyo markets) cannot therefore be consumed as the sole source of energy to humans. The low level of energy could be attributed to low levels of crude protein and high levels of moisture in the samples. In order to derive full nutritional potentials of these fish, they should be consumed with fat-giving foods since low energy may lead to low birth weight and increased infant mortality.

5. Conclusion

The microbiological and physiochemical analysis of Tilapia fish from Uyo and Itu markets revealed that samples from Itu market contain high microbial load but are nutritionally richer than samples from Uyo market. This means that the samples from itu have high contaminants and unacceptable levels of both bacterial and fungal isolates. Considering the public health implications of the poor bacteriological and mycological qualities of these fish samples, particular attentions should be paid to their safety through proper processing, storage and handling procedures as most of these organisms are from the handlers.

From the result obtained in this study, it is therefore recommended that people should be educated on the unhealthy implication of water pollution as this goes a long way in contaminating the aquatic animals. Proper

processing, storage, and handling procedures should be cultivated. Adequate processing methods before consumption should be employed. Fish that have shown signs of spoilage should not be sold to the public so as to boost the health standard of the populace. The populace should also be educated on the proper measures to be embarked in sewage and effluents disposal and not to the rivers as this would pollute the water and cause health risk.

Correspondence to:
Iheanyi O. Okonko
Department of Microbiology,
University of Port Harcourt, Choba,
PMB 5323 Port Harcourt, Rivers State, Nigeria;
E-mail:mac2finney@yahoo.com;
iheanyi.okonko@uniport.edu.ng
Tel.: +234 803 538 0891

REFERENCES
1. Adams, M.R, and Moss. M.O. (1995). Food Microbiology, Royal Society of Chemistry. Science Park, Cambridge pp17, 122 -122
2. Adebayo-Tayo AC; Odu NN; Michael MU;Okonko IO. Multi-Drug Resistant (MDR) Organisms isolated from Sea-foods in Uyo, South-Southern Nigeria. Nature and Science 2012a; 10(3): 61-70
3. Adebayo-Tayo BC, Adegoke AA and Akinjogunla OJ. 2009. Microbial and physico-chemical quality of powdered soymilk samples in Akwa Ibom, South Southern Nigeria. African Journal of Biotechnology, 8 (13): pp. 3066-3071
4. Adebayo-Tayo BC, Onllude AA, Ogunjobi AA. 2006. Bacteriological and Proximate Analysis of Periwinkle from two different creeks in Nigeria. *World Applied Science Journal 1* (2) 87-91.
5. Adebayo-Tayo BC; Odu NN;Anyamele LM; Igwiloh NJPN;Okonko IO. Microbial Quality Of Frozen Fish Sold In Uyo Metropolis. Nature and Science 2012b; 10(3): 71-77
6. Adeleke EO, Odelola HA (1997). Plasmid profile of multiple drug resistant strains of *Staphylococcus aureus*. Afr. J. Med. Sci. 26: 191-121.
7. Agbu, A.A., Alariba, H.C., Singh, K., and Adesiyun, A.A. (1998) Bacteriological studies and chemical analysis of public well water in Samaru and Zaria city in Northern Nigeria. Journal of Microbiology, 8 (1-2): 88-98.
8. Allen JL, Cowan ME (1997). Monitoring outbreaks of methicillinresistance *Staphylococcus aureus.*

Use of a commercial database and personal computer B.J. Biomed. Sci. 54: 10-12.
9. Amusa NA, Ashaye OA, Aiyegbayo AA, Oladapo MO, Oni MO, Afolabi OO (2005). Microbiological and nutritional quality of hawked sorrel drinks (Zoborodo) (the Nigerian locally brewed soft drinks) widely consumed and notable drinks in Nigeria. Food, Agric. Environ. 3: 47-50.
10. Arannilewa ST, Salawu SO, Sorungbe AA, Ola-Salawu BB (2005). Effect of frozen period on the chemical, microbiological and sensory quality of frozen tilapia fish (*Sarotherodun galiaenus*). Afr. J. Biotechnol. 8: 852-855.
11. Arannilewa ST, Salawu SO, Sorungbe AA, Ola-Salawu BB (2006). Effect of frozen period on the chemical, microbiological and sensory quality of frozen Tilapia fish (Sarotherodun galiaenus). Nutr. Health 18(2):185-92.
12. Association of Official Analytical Chemists (AOAC) (1990). *Official Methods of Analysis.* 14th Edn. Association of Analytical Chemists. Washington DC.
13. Ayres JC. 1993. The physiology of the microbial spoilage of foods. pp23: 206 – 215.
14. Baixas-Nogueras S, Bover-Cid S, Veciana-Nogues MT, Marine-Font A, and Vidal-Carou MC. 2005. Biogenic amine index for freshness evaluation in iced Mediterranean hake (*Merluccius merluccius*). J Food Prot 68:2433–2438.
15. Bergdoll MS (1981). Bacillus cereus, food borne disease. Clin. Microbiol. Newsl. 3: 85-87.
16. Briggs, G.N. and D. Colloway. 1979. Nutritional and physical fitness. 10th Edn, W.B. Sanders Company London, pp:401-402.
17. Cheesbrough M (2000). District Laboratory Practice in Tropical Countries. Low price ed. Cambridge University Press, pp. 310-331.
18. Dalgaard P, Madsen HL, Samieian N, and Emborg J. 2006. Biogenic amine formation and microbial spoilage in chilled garfish (*Belone belone belone*)— effect of modified atmosphere packaging and previous frozen storage. J Appl Microbiol 101:80–95.
19. Dongo EI, Ayodele SM (1997). Fungal air spora of some selected localities in Delta State University. Abraka, Delta State. Bull. Sci. Assoc . Niger., 21: 9-14.
20. Doyle EM. 2007. FRI BRIEFINGS: Microbial Food Spoilage: Losses and Control

Strategies. *A Brief Review of the Literature.* Food Research Institute, University of Wisconsin–Madison http://fri.wisc.edu/docs/pdf/FRI_Brief_Microbial_Food_Spoilage_7_07.pdf

21. Eknath AE, Bentsen HB, Ponzoni RW, Rye M, Nguyen NH, Thodesen J, Gjerde B (2007). Genetic improvement of farmed tilapias: Composition and genetic parameters of a synthetic base population of *Oreochromis niloticus* for selective breeding. Aquaculture, 273: 1- 14.

22. Emborg J, Laursen BG, and Dalgaard P. 2005. Significant histamine formation in tuna (*Thunnus albacares*) at 2°C—effect of vacuum- and modifiedatmosphere- packaging on psychrotolerant bacteria. Int J Food Microbiol 101:263–279.

23. Fishwick, W. G. and Wright, A. Y. (1977). Comparison on methods for the extraction of plant Lipids. *Phytochemistry* 16: 1507 -1510

24. Foster T (1996). Staphylococcus. In: Barron's Medical Microbiology, 4th ed., University of Texas Medical Branch. ISBN 0-9631172-1-1

25. Gram L and Dalgaard P. 2002. Fish spoilage bacteria— problems and solutions. Curr Opin Biotechnol 13:262–266.

26. Haugen JE and Undeland I. 2003. Lipid oxidation in herring fillets (*Clupea harengus*) during ice storage measured by a commercial hybrid gas-sensor array system. J Agr Food Chem 51:752–759.

27. Hood MA, Ness GE, Blake NJ (1983). Relationship among fecal coliforms, *Escherichia coli* and *Salmonella* spp. in shellfish. Appl. Environ. Microbiol. 45(1): 122-6.

28. Hozbor MC, AI Saiz, MI Yeannes and R Fritz. 2006. Microbiological changes and its correlation with quality indices during aerobic iced storage of sea salmon (*Pseudopercis semifasciata*). LWT - Food Science and Technology, 39(2): 99-104

29. IIondu EM, Iloh AC (2007). Inhibition of three fungal isolates from sorrel drinks ("zobo") using hurdle technique. World J. Agric. Sci. 3(3): 339-343.

30. Jay JM (1978). Modern Food Microbiology 2nd Ed. Van Nostran Reinhold. C. New York, pp. 321-324.

31. Jeyasekaran G, Ganesan P, Anandaraj R, Jeya Shakila R, Sukumar D (2006). Quantitative and qualitative studies on the bacteriological quality of Indian white shrimp (*Penaeus indicus*) stored in dry ice. J. Food Microbiol. 23(6): 526-533.

32. Jolt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST. 1994. Bergey's manual of systematic bacteriology, 9th edn. Williams & Wilkins Co. Baltimore, Maryland, p786

33. Klein E, Smith DL, Laxminarayan R (2007). Hospitalizations and Deaths Caused by Methicillin-Resistant *Staphylococcus aureus* in United States, 1999–2005. Emerg. Infect Dis. 13(12): 1840-1846.

34. Koutsoumanis K, Nychas GJ (2000). Application of systemic experimental procedure to develop a microbial model for rapid fish shelf life predictions. Int. J. Food Microbiol. 60(2-3): 171-184.

35. Li SF, Cai WQ (2008). Varieties characteristics and cultured techniques of GIFT strain of Nile tilapia (*Oreochromis niloticus*). Scientific fish aquiculture, 5: 21-22.

36. Liu S, W Fan, S Zhong, C Ma, P Li, K Zhou, Z Peng and M Zhu. 2010. Quality evaluation of tray-packed tilapia fillets stored at 0°C based on sensory, microbiological, biochemical and physical attributes. African Journal of Biotechnology, 9(5): 692-701

37. Nester, E.W., C.E. Roberts and M.T. Nester, 1995. Microbiology, A Human perspective WM.C Brown publishers. Oxford, England.

38. Odunfa SA (1988). Microbiological quality of yoghurt and milk drink samples in Ibadan, a Nigerian City. J. Agric. 2: 43-46.

39. Okhuoya JA, Ayanlola JO (1986). Air spora of selected localities in Benin City. Nigeria. Niger. J. Pure Appl. Sci. 1: 75-83.

40. Okonko IO, Ogun AA, Adejoye OD, Ogunjobi AA, Nkang AO, Adebayo-Tayo BC. 2009. Hazards analysis critical control points (HACCP) and Microbiology qualities of Sea-foods as affected by Handler's Hygiene in Ibadan and Lagos, Nigeria. African Journal of Food Sciences, 3(1):035-050

41. Okonko, L.O., A.A. Ogunjobi, E.A. Fajobi, B.A. Onaja, E.T. Babalola, A.O. Adedeji (2008). Comparative Studies and Different Assessment of Ready-to-Eat (RTE) Frozen Sea Foods Processed in Ijola-Olopa Lagos State, Nigeria. J. Afr. BioTechnol. 16:2898 – 2901.

42. Okuma K, Iwakawa K, Turnidge J (2002). Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J. Clin. Microbiol. 40(11): 4289-4294.

43. Olafsdottir G, Jonsdottir R, Lauzon HL, Luten J, and Kristbergsson K. 2005. Characterization of volatile compounds in chilled cod (*Gadus morhua*) fillets by gas chromatography and detection of quality

indicators by an electronic nose. J Agr Food Chem 53:10140–10147.

44. Osborne, D. and Voogt (1978). Analysis of nutrients in foods. Academic press, London N. Y. San Francisco. pp100-159.

45. Osuntogun B, Aboaba OO (2004). Microbiological and physico-chemical evaluation of some non-alcoholic beverages. Pak. J. Nutr. 3(3): 188-192

46. Oyeleke SB, Manga SB. 2008. Essentials of Laboratory Practicals in Microbiology. Tobest Publisher, Minna. Nigeria, pp. 36-75.

47. Potter NN (1983). Food science. 3rd ed. AVI Publishing company incorporated, Westport.

48. Prescott LM, Harley JP, Klein DA (2005). Microbiology (6th edn.). McGraw-Hill Companies, Inc., New York.

49. Scott K (2002). Epidermiological and Microbiological Characterization of Infection causes by *Staphylococcus aureus* with Reduced Susceptibility to Vancomycin, United State, p. 1997.

50. Sil S, Joseph J, Kumar KA (2008). Changes in biogenic amines during iced and ambient temperature storage of tilapia. J. Sci. Food Agric. 88: 2208-2212.

51. Stewart FS (1974). Bigger's Bacteriology and Immunology for students of medicine 9th edn. ELBS and Bailliere, Tindal and Casell.

52. Yagoub S.O. 2009. Isolation of *Enterobacteriaceae* and *Pseudomonas spp.* from raw fish sold in fish market in Khartoum state. Journal of Bacteriology Research, 1(7): 085-088

53. Yanar Y, Celik M, Akamca E (2006). Effects of brine concentration on shelf-life of hot-smoked tilapia (*Oreochromis niloticus*) stored at $4^{O}C$. Food Chem. 97: 244-247.

54. Zapata AG, A. Chiba A, Vara A. 1996. Cells and tissues of the immune system of fish. In: The Immune system: Organism, Pathogen and Environment. Fish Immunology Series (Iwama G, Nakanshi T eds.) New York, Academic Press, p1-55.

2/13/2012