The Pharmaceutical and Chemical Journal, 2018, 5(2):119-128

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Microbial and Chemical Evaluation of Parts of Fresh and Smoked Tilapia (*Oreochromis niloticus*) and Catfish (*Clarias gariepinus*)

Iyama William Azuka, Uchendu Goodnesschinwe

Department of General Studies, School of Foundation Studies, Rivers State College of Health Science and Technology, Port Harcourt, Nigeria

Abstract This study was based on the determination of microbial and chemical evaluation of different parts of fresh and smoked Tilapia (*Oreochromis niloticus*) and Catfish (*Clarias gariepinus*). This was achieved by the study of the microflora of different parts of catfish and tilapia (head, flesh and tail) to determine the microbial diversity of these parts (tissues) and their potential hazards. The pH, moisture content and microbial counts of bacteria and fungi were determined (enumeration of coliforms staphylococci) using pH meters, weighing and plate count agar. Results show high moisture content on the heads of both fish types and the pH of the head samples were more neutral-alkali values of range 7.01-7.82. The highest bacterial count was on the heads and minimum of 2.3×10^3 Cfu/g and maximum of 9.6×10^5 Cfu/g. Similarly, the fungi count was more on the head but relatively lower than that of the bacteria counts. The minimum fungi count of 2.5×10^3 Cfu/g and maximum of 6.3×10^4 Cfu/g were recorded on the head. On the average, bacteria and fungi counts were least by the tails. The following organisms were identified, pseudomonas, Escherichia coli, shigella, salmonella, staphylococcus, micrococcus, proteus, bacillus, seretia and citrobacter. Analysis was done using bar charts and line graphs to show trend and relationships.

Keywords fresh fish, smoked fish, tilapia, catfish, hot-smoking

Introduction

Oreochromis niloticus commonly called tilapia comes from the *tilapiini cichlid* tribe of *tilapiini* which inhabits a variety of freshwater habitats including shallow streams, ponds, rivers and lakes. Some common examples of known cichlid species belonging to the genus tilapia are spotted tilapia (*Tilapia Mariae*), Okavango tilapia (*Tilapia Ruweti*), Otjikoto tilapia (*tilapia guinansana*) and Guinean tilapia (*Tilapia Guineensis*). According to Akinwumi and Adegbehingbe [1], fish is an essential source of protein especially in developing countries like Nigeria. Fish also has curative properties such as reduction in asthma cases, coronary heart diseases, goiter and even cancer [2]. Fish is another good source of vitamins and essential minerals [3]. Fish is a relatively cheaper meat protein hence a major source of animal, protein for the food diet of our people.

Fish easily deteriorates especially at high ambient temperatures so deserves preservation. The major preservation methods available are either freezing, salting, sun-drying, oven-drying, fermentation or smoking [4-5].

According to Akinwumi and Adegbehingbe [1], smoked fish are usually hawked around without putting into consideration their microbial load and hence contamination. In this vein, the main objective of this study was to isolate the available microorganisms in fresh and smoked fish of tilapia and catfish making comparison where necessary. The tilapiine cichlids are the major interest for fish farmers especially the *Oreochromis* and S*arotherodon* species collectively called tilapia. This is called due to its size, rapid growth rate and palatability. They are good sources of protein and common among artisanal and commercial fisheries as were originally found in Africa. They

are also found in outdoor fishfarms in the tropics (Nigeria), the Philippines and Indonesia in fresh water lakes. Research has shown that Tilapia is prolific breeder but the males are the most commercially grown. Tilapias seldom survive in temperate climates as they require warm aquatic temperature.

However, the pure strain of the blue tilapia, *Oreochromis aureus* has the greatest cold tolerance. This enables them to invade temperate habitats and could disrupt native ecologies in temperate zones. These above species has widely spread beyond their points of introduction in many fresh and brackish tropical and subtropical habitats. Fish serves as food to man being a major source of protein. However, tilapia like other fish species carries millions of different bacteria species on their outer surfaces and in their guts. The guts contain huge numbers of bacteria which can easily contaminate the cavity when eviscerated carelessly. The breakdown of the cavity allows for penetration to all parts of the flesh known as auto-digestion.

Tilapia has very low level of mercury (Hg) as they are fast growing, lean and short-lived with a primarily vegetarian diet, hence does not accumulate mercury found in many prey. Tilapia is low in saturated fat calories, carbohydrates and sodium, so a good source of protein [1, 6]. Tilapia contains micronutrients such as phosphorus, ricacin, vitamin B_{12} and potassium.

The flora of freshly caught fish is a reflection of the microbial quality of the aquatic environment. Though, the slime gills and guts are the sites easily colonized by microorganisms. These microorganisms incidentally are the major causes of spoilage of most sea food products [7]. The common genera of microorganisms associated with various fish tissues are *Pseudomonas, Alcaligenes, Micrococcus, Flavobacterium, Coryne Bacterium, Serratia, Vibrio, Bacillus Aeomonas, Lactobacillus, Brevibacterium and Streptococcus*. Microbial population increases by contamination according to the nature, number and kind of materials in contact. Generally, the bacteria found on fishes in temperate regions are primarily psychrophiles while those from the tropics are mesophiles. Microbial load is usually low during starvation period and high soon after feeding.

Fish spoilage is mainly due to the microorganisms associated with the fish in equilibrium while the fish is alive but soon invade the tissues and other organs when dead usually facilitated by autolytic activity. Therefore, on invasion, the bacteria use the fish as a source of nutrient following autolysis. Fish therefore is subject to spoilage after capture due to high moisture content (about 80% reason for spoilage).

Therefore, caught fish need to be preserved as not all can be consumed immediately. Some notable preservation methods are smoking especially due to lack of adequate storage facilities [8]; use of chemical such as organic acids, esters and sodium chloride; drying, salting and pickling. The chemical preservative often acted synergistically with other unfavourable physiological conditions like reduced water activity of the sea food and decreased pH value [8].

Materials and Methods

Live Tilapia (*Oreochromis niloticius*) was bought from the Wimpey Market, mile 4 Diobu, Port Harcourt. This was immediately transported to the laboratory in plastic containers of fresh water. The samples were average length 15.15cm and 2.0cm in diameter weighing 5g and 10g respectively.

For microbial analysis, a 10g portion was obtained aseptically from the head (pooled eye, opercula and gills) or flesh (skin and tissues) and tail using sterile scapel and blended with a pre-sterilized moulinex blender with 90ml of 0.1% peptone water. This was quickly followed by tenfold serial dilution of each sample to 10^{-6}. From each dilution, 0.1ml aliquot was spread plated on pre-poured plates of plate count Agar (PCA) and potato Dextrose Agar (PDA) in duplicate using spread plate technique and incubated at 37^{o}C or 28^{o}C for 18-24 hours or 3-4 or 4-5 days respectively. Finally, the plate count Agar was used for enumeration of total viable counts.

Similarly, potato dextrose Agar for fungal counts and other selective media such as macConkey Agar and Mannitol Salt Agar were used for enumeration of coliforms and staphylococci.

At the end of the incubation, plates showing counts of 30-300 colonies were enumerated. The mean counts from the duplicate plates were determined and recorded as colony forming units or grams. Gram staining was done with relative gram reactions. They were then purified by sub-culturing and stored in PCA slants in a refrigerator and later for biochemical tests. The isolates were then identified using cultural, morphological and biochemical/physiological characteristics.

The motility media used for this test was prepared by dissolving 10g tryptone, 0.5g Agar and 5g NaCl in 100ml distilled water and dispensed in 10ml amount into test sterilized by autoclaving at 121 ^{o}C for 15 minutes and left to set in vertical position. These were stab-inoculated with a sterile straight wire to a depth about half-way into the medium and incubated at 37 ^{o}C for 18-24 hours.

Catalase and oxidase biochemical tests were carried out. A loopful of the test organism from an 18-24hours old culture was placed on a microscope slide and 2 drops of 3% hydrogen peroxide was added to it, where effervescence shows a positive test of enzyme catalase production [9]. For oxidase, a loopful of the test organism was transferred with a sterile loop onto a whatmann No.1 filter paper drained with 4 drops of Kovac's reagent (1% aqueous solution of tetramethylparaphenylene diaminechloride). Hydrogen sulphide production was achieved using the Kligler's triple sugar iron Agar. Inoculation was done by stabbing the butt and streaking the slant and incubated at 37 ^{o}C for 24 hours.

Fermentation of sugars was done when the isolates were inoculated into basal medium containing test sugars. This medium was then compounded by mixing 10% sugar, 0.5% NaCl, 1% peptone and 1.25ml of bromothylmol blue indicator and pH adjusted to 7.0 and 10ml portion was dispersed into test tubes.

Fungal isolates were identified using morphological and microscopic characteristics. The features used for cultural features included colour, elevation and appearance and microscopic identity including the presence or absence of cross walls (septa) conidia and spore arrangements. These were done by teasing the isolates in lactophenol cotton blue on a slide and examined microscopically. To determine the pH, a 10g portion of each sample type i.e head, flesh, tail was blended in 20 ml distil water and then measured using pH meter, equip-tronics, model EQ-610. The sample solutions were prepared by homogenizing the sample in distilled water as to form slurry.

Results and Discussion

Tables 1 and 2 show both the moisture content and pH values for fresh and smoked tilapia and catfish samples. Total viable counts for different parts of fresh and smoked tilapia are shown in Tables 3 and 4 respectively.

Similarly, Tables 5 and 6 illustrates values recorded for total viable counts for different parts of fresh and smoked catfish. Bacterial count for both fresh and smoked tilapia showing % variations on the head, flesh and tail is shown on Table 7. Similarly, the fungi count for both fresh and smoked tilapia showing % variations for head, flesh and tail is reported on Table 8.

The bacterial count for both fresh and smoked catfish showing % variation between fresh and smoked for head, flesh and tail is on Table 9. Finally, the fungi count for both fresh and smoked catfish showing % variation between the fresh and smoked sample for the head, flesh and tail is reported in Table 10.

Table 1: Moisture content and pH for Fresh and smoked Catfish

Storage Time	**Sample**	**Moisture Content**	**pH**
Day 0	Head	71.1	7.8
	Fresh	70.3	7.0
	Tail	68.0	6.72
Smoked Sample			
Storage Time	Sample	Moisture Content	pH
2 Day after	Head	20.70	7.01
	Flesh	20.83	6.65
	Tail	20.20	6.63
Smoked Sample			
Storage Time	Sample	Moisture Content	pH
4 Day after	Head	23.92	7.82
	Flesh	23.96	7.65
	Tail	23.85	6.81

Table 2: Moisture content and pH for fresh and smoked Tilapia sample

Storage Time	**Sample**	**Moisture Content**	**pH**
Day 0	Head	20.99	7.02
	Fresh	20.45	6.69
	Tail	20.70	6.66
Smoked Sample			
Storage Time	Sample	Moisture Content	pH
2 Day after	Head	22.77	7.78
	Flesh	22.52	7.58
	Tail	22.45	6.63
Smoked Sample			
Storage Time	Sample	Moisture Content	pH
4 Day after	Head	23.86	7.06
	Flesh	23.92	6.78
	Tail	22.99	6.68

Table 3: Total viable counts for different parts of fresh tilapia

Microbial flora	**Sample**	**Cfu/g**
Bacterial	Head	$9.6x10^5$
	Fresh	$6.0x10^4$
	Tail	$3.3x10^3$
Fungi	Head	$5.0x10^4$
	Flesh	$3.0x10^3$
	Tail	$2.5x10^3$

Table 4: Total viable counts for different parts of smoked tilapia

Microbial flora	**Sample**	**Cfu/g**
Bacterial	Head	$3.3x10^3$
	Fresh	$2.0x10^2$
	Tail	$1.6x10^2$
Fungi	Head	$2.5x10^3$
	Flesh	$1.8x10^3$
	Tail	$1.5x10^2$

Table 5: Total viable counts for different parts of fresh Catfish

Microbial flora	**Sample**	**Cfu/g**
Bacterial	Head	$9.2x10^4$
	Fresh	$7.5x10^3$
	Tail	$5.6x10^3$
Fungi	Head	$6.3x10^4$
	Flesh	$4.5x10^3$
	Tail	$3.4x10^2$

Table 6: Total viable counts of different parts smoked catfish

Microbial flora	**Sample**	**Cfu/g**
Bacterial	Head	$2.3x10^3$
	Fresh	$1.9x10^3$
	Tail	$1.6x10^2$
Fungi	Head	$2.0x10^4$
	Flesh	$1.5x10^3$
	Tail	$1.3x10^2$

Table 7: Bacterial count for both fresh and smoked Tilapia

Sample	**Fresh**	**Smoked**	**% Variation**
Head	$9.6 x10^5$	$3.3 x10^3$	99.66
Flesh	$6.0 x10^4$	$2.0 x10^2$	99.67
Tail	$3.3 x10^3$	$1.6 x10^2$	95.15

Table 8: Fungi count for both fresh and smoked Tilapia

Sample	Fresh	Smoked	% Variation
Head	5.0×10^4	2.5×10^3	95
Flesh	3.0×10^3	1.8×10^3	40
Tail	2.5×10^3	1.5×10^2	94

Table 9: Bacterial count for both fresh and smoked Catfish

Sample	Fresh	Smoked	% Variation
Head	9.2×10^4	2.3×10^3	97.50
Flesh	7.5×10^3	1.9×10^3	74.67
Tail	5.6×10^3	1.6×10^2	97.71

Table 10: Fungi count for both fresh and smoked Catfish

Sample	Fresh	Smoked	% Variation
Head	6.3×10^4	2.0×10^4	68.25
Flesh	4.5×10^3	1.5×10^3	66.67
Tail	3.4×10^2	1.3×10^2	61.76

Figures 1 and 2 illustrate pH and moisture content for catfish at 0, 2 and 4 day periods. The pH of both fresh and smoked tilapia is on Figure 3 whereas the moisture content is on Figure 4. The total microbial load for fresh and smoked tilapia samples for days 0, 2 and 4 days after are illustrated in Figures 5 and 6 respectively. Figures 7 and 8 depict the relationships existing between total viable counts for fresh and smoked catfish.

Figure 9 shows the bacteria and fungi counts for fresh and smoked tilapia whereas Figure 10 is graphical relationship between the bacteria and fungi counts for both fresh and smoked catfish. Results show that there are various types of microorganisms associated with fresh and smoked tilapia as well as fresh and smoked catfish [1]. These are mostly bacteria and fungi. Results also indicate the prevalence of bacteria in aquatic environment compared to the fungi population. Smoking according to Efiuvwevwere and Ajiboye [10] posits that shelf life of fish is extended due to reduced moisture content and the effects of imported phenolic compounds.

Potential pathogenic bacteria isolated from the fish samples are *Pseudomonas, Micrococcus, Staphylococcus, Salmonella, Escherichia coli, Bacillus Seretia, Shigella and Streptococcus.* Similarly, the fungal isolates included *Aspergillus, Rhizopus Penicillium Fusarium, Mucor and Candia.* Most of these organisms are associated with food poisoning infections and typhoid fever in humans, *shigellosis* food infection in human and the presence of *Aspergillus* species reveals possible production of Aflatoxins which are carcinogenic. The poikilotherm nature of fish indicates the form of bacteria that can thrive in a wide range of temperature. This is exemplified by the microflora of temperate water fish dominated by psychrotrophic Gram-negative, rod-shaped bacteria like *pseudomonas* and *Moraxella* [11].

The discoloration of fish during spoilage arises from the oxidation of the oxygen transporters in fish blood *i.e* myoglobin to metamyoglobin during frozen storage or from prolonged or unnecessary exposure of the fish to air which is oxidative [12-13].

The prevalence of bacteria in fish spoilage was supported by the pressure of psychrotrophs which can tolerate cold temperature and grow at 0^oCbut grow optimally at about 25^oC [11].

In fresh fish, the specific spoilage bacteria include *Shewanella putrefaciens* and *Pseudomonas spp*. [11, 14]. The percentage variation in both fungi and bacterial count for fresh and smoked fish (Tilapia and catfish) exceed 50% except for the flesh which was 40%. This indicates high degree difference in spoilage for fresh fish compared to smoked type. This is exemplified by the 95-99% range in variation observed for bacteria count for both fresh and smoked tilapia at the head, flesh and tail. This analysis has also shown that tilapia fish has a relatively lower bacterial count and fungi count than catfish. Efiuvwevwere and Iweanoge [15] confirmed similar results as moisture content reduced after smoking but pH increased further.

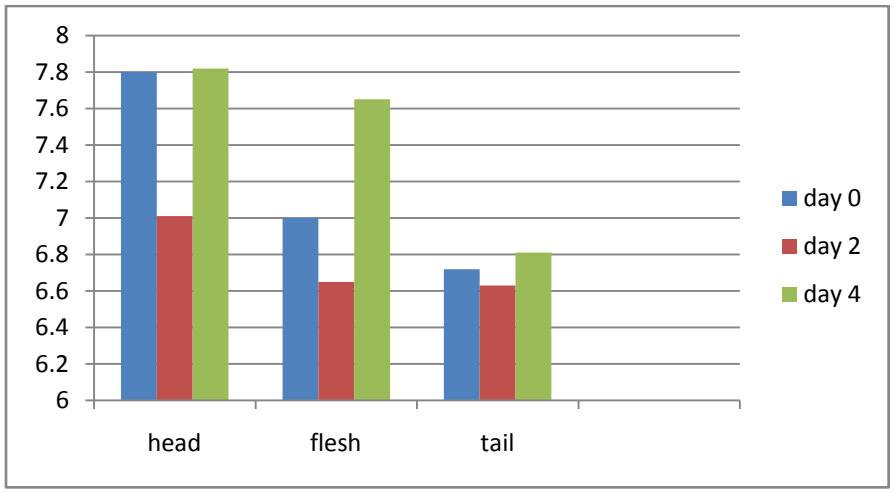

Figure 1: pH of catfish for days 0, 2, 4

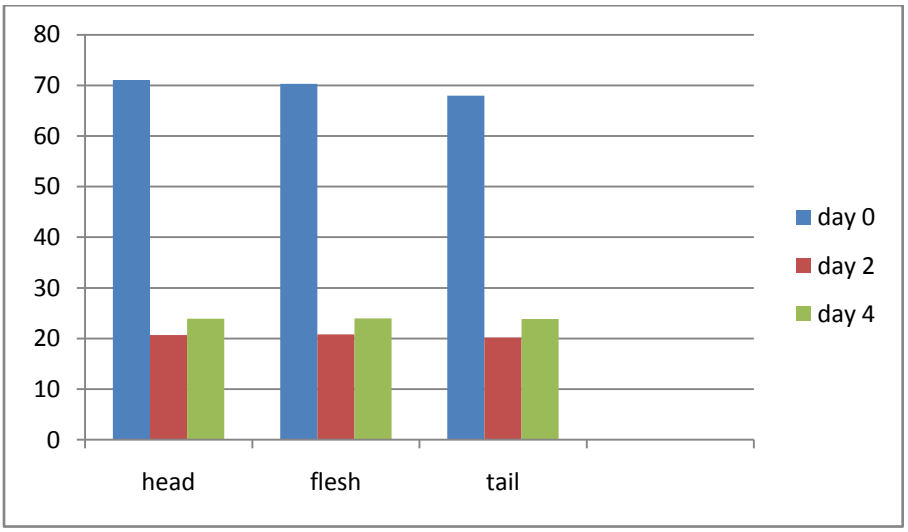

Figure 2: Moisture contents for days 0, 2, 4.

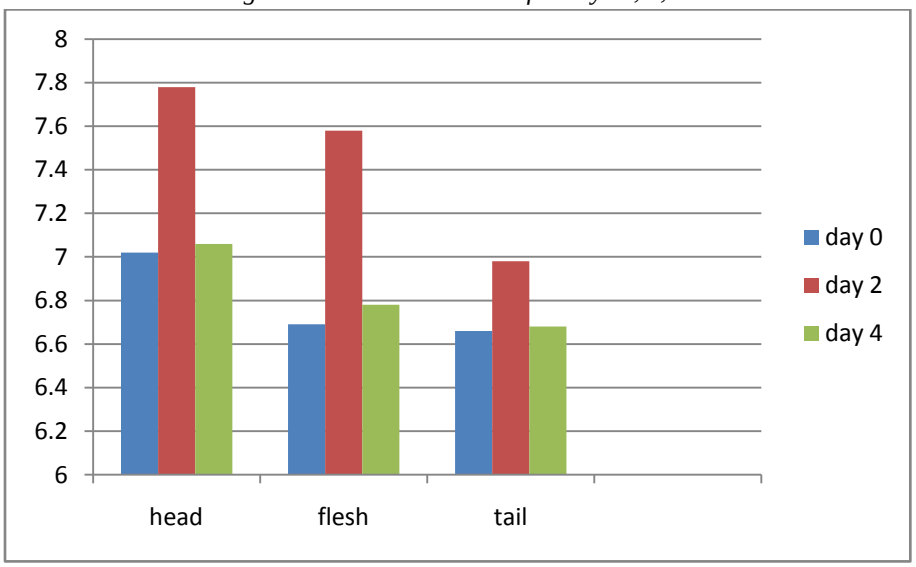

Figure 3: pH of fresh and smoked fish

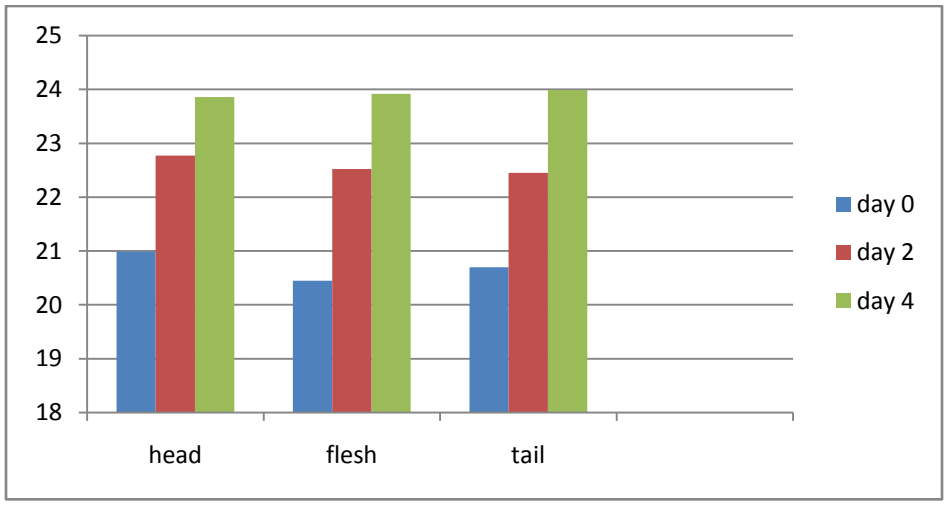

Figure 4: Moisture content of fresh and smoked Tilapia

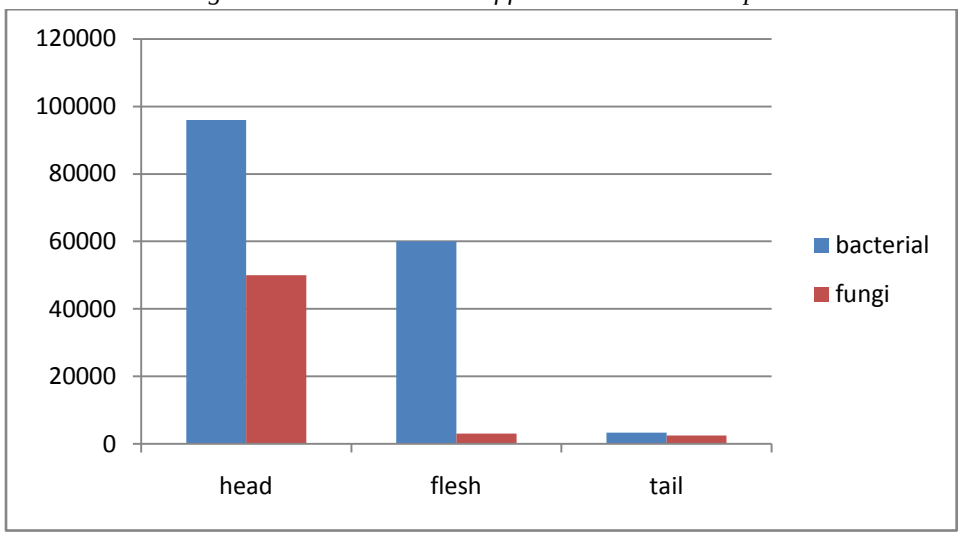

Figure 5: Total variable counts for fresh tilapia

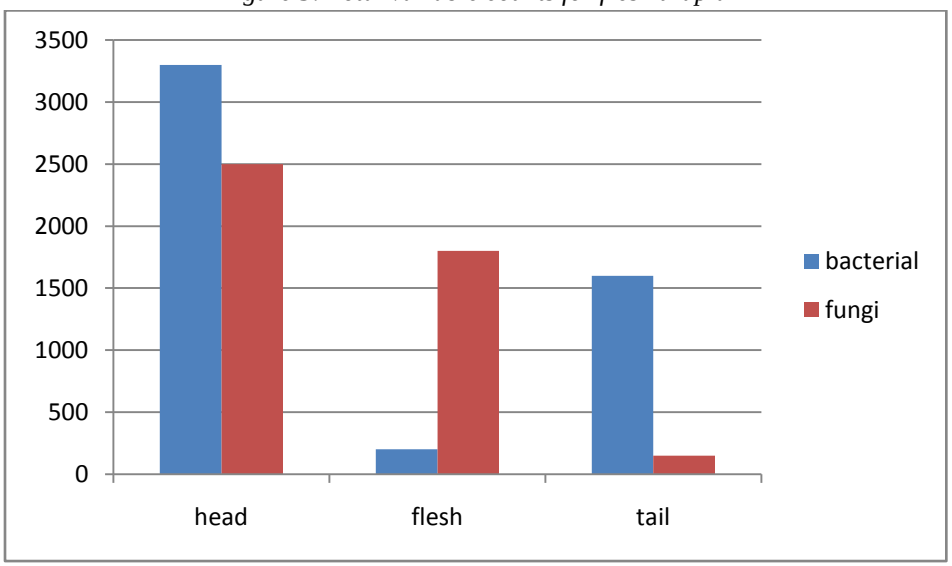

Figure 6: Total Viable Counts for Smoked Tilapia

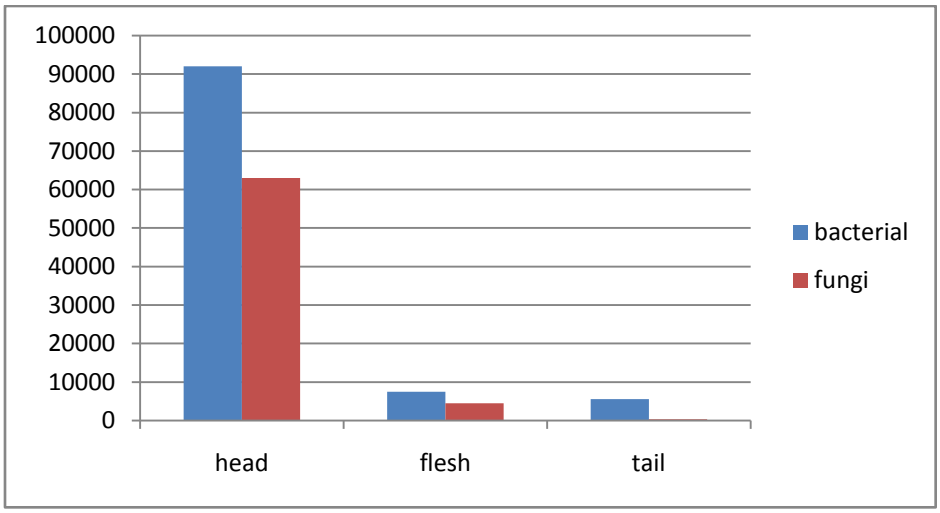

Figure 7: Total Viable Counts for fresh Catfish

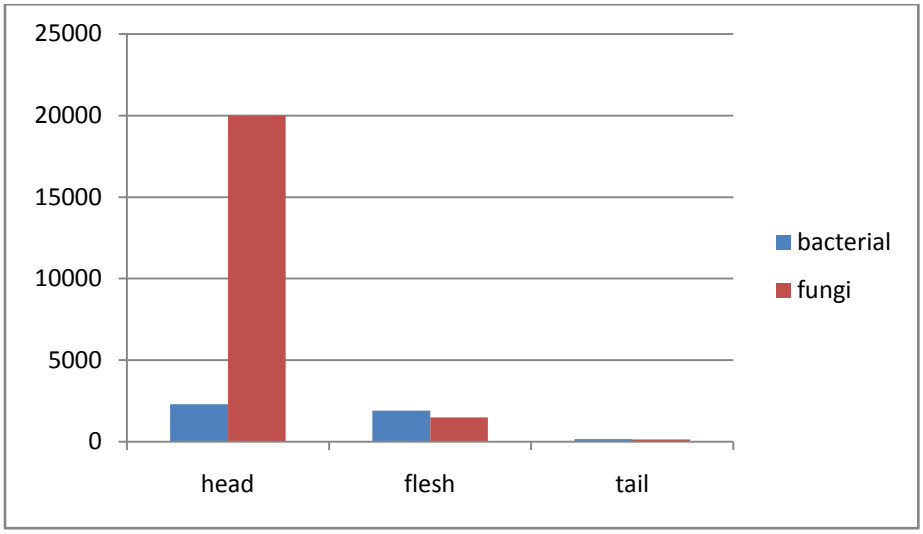

Figure 8: Total Viable Counts for Smoked Catfish

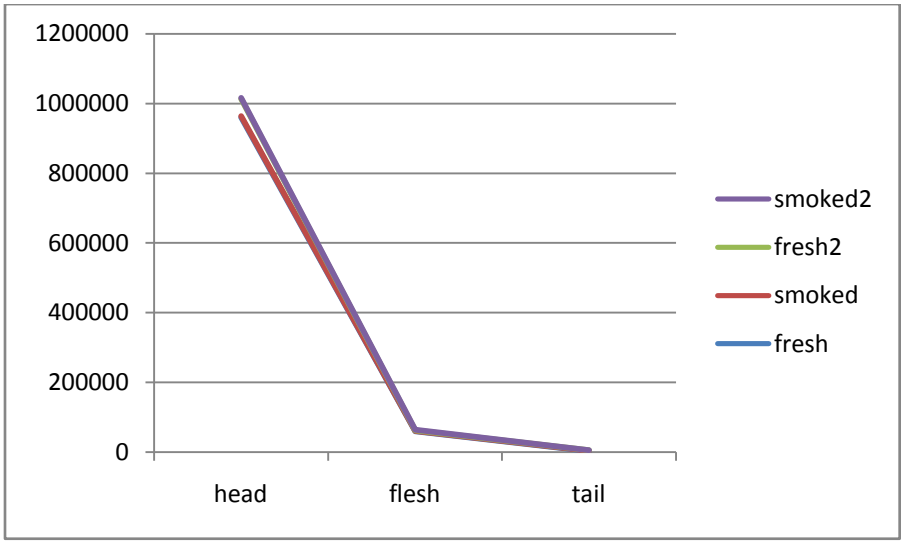

Figure 9: Bacteria and fungi counts for both fresh and smoked Tilapia

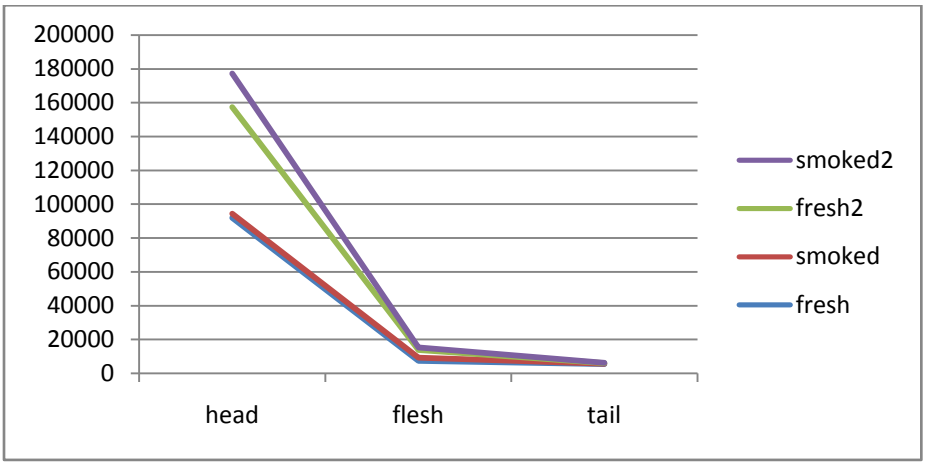

Figure 10: Bacteria and fungi counts for both fresh and smoked Catfish

The moisture content for fresh catfish recorded the highest value of 71.1 at the head and pH of 7.8 units and least at the tail of 68 and pH of 6.72 units. For smoked catfish, a moisture content of 23.85 was recorded at the tail after 4 days of smoking-drying. This was really high relative to those recorded by Akinwumi and Adegbehingbe [1] in all their stations. This maybe attributed to the nature of the waterbody whether fresh or brackish [15].T he moisture content for tilapia [day 0] was 20.99 while the pH was 7.02 on the head being the highest. The moisture content was least for smoked catfish after 2 days at 20.20 by the tail. This is probably due to the smaller surface area of the tail hence easier to eliminate water. The total bacteria count for fresh tilapia was highest at the head (9.6×10^5 Cfu/g) as well as the fungi count (5.0×10^4 Cfu/g). The basic explanation is the anatomy of the head which gives room for faster spoilage and decay which increases total microbial count. This was similar for the smoked tilapia though both viable counts for bacteria and fungi were less compared to the fresh. This cannot be over emphasized as smoking reduces the prevalence of microbial load (Bacteria count for the head-$33x10^3$Cfu/g; fungi count-$25x10^5$ Cfu/g).Similarly, the bacteria count for catfish was highest at the head ($9.2x10^5$) as well as fungi ($6.3x10^5$) but lower for smoked catfish which were $2.3x10^5$ and $2.0x10^5$[bacteria and fungi] counts at the head respectively. These results were in consonance with those obtained for samples from sampling stations in Ondo State [1].

The high level of microorganisms found in parts of the fresh and smoked fish have also been reported in some other fish species [16-17]. The variation or trend in microbial counts can also emanate from the type of water as reported by Alexander and Austin [15] from polluted water bodies and Teugels and Audenerde [18] on fresh and brackish water fishes of West Africa. According to Salihu-lasisi, Akpabio and Ogunsola [19], processed fish should be smoked at high temperature which can reduce moisture content hence discouraging the growth of micro-organisms being the agents of food and fish spoilage.

Conclusion

Smoking reduces the total plate count of day zero (0) smoked samples. There was relatively reduced moisture content after smoking but conversely the pH increased. Hot smoking reduces some of the fat and oils contained in the fresh fishes rendering the fish low in calories. There is more bacterial count at the heads of these fishes compared to the flesh and tails. This is also corroborated by the fungi count. This may have been due to the bones and gills found on the head making even smoking and drying slower. The result has also indicated that there is comparatively higher bacteria count than fungi count on the three parts so studied for both kinds of fish indicating the prevalence of more fish spoilage by bacteria.

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