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Microbial Load (Bacteria, Coliform and Mould Count/Flora) of Some Common Hot Smoked Freshwater Fish Species Using Different Packaging Materials

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ABSTRACT

Three different packaging materials of (37 cm × 25 cm) size (Sealed Transparent Polythene Bag (STPB) Sealed Paper Bag (SPB) (Brown envelope), Open Mouth Polythene Bag (OMPB) (Black incolour)) were used for *Oreochromis-niloticus* (O), *Clariasgariepinus* (C) and *Mormyrusrume* (M). Twenty fish samples per species (averaging 250 gm) were hot smoked dried whole for 36 hours at an average temperature of 100˚C. Packaged hot at the rate of 6 fishes per package for each species (three packs for each packaging treatment *i.e*. 18 pieces were packed while the remaining 2 pieces were used for initial bacteria load and microbial load). Microbial load (Total Viable Count (TVC), Total Coliform Count (TCC) and Total Fungi Count (TFC)) for the fresh fish was initial hot smoked and finally at the end of 12 weeks was monitored. The TVC (bacterial load) of *O. niloticus* dropped from (10.6 - 8.4) × 10^4 (fresh state-hot smoked) and *M. rume* (9.8 - 7.0) × 10^4, while *C. gariepinus* slightly increased from (12.4 - 12.6) × 10^4. After hot smoking, highest TVC of 8.6 × 10^4 (OMPBC), 8.3 × 10^4 (SPBC) and 8.2 × 10^4 (STPBC) was recorded in *C. gariepinus* among the 9 packaging at 12 weeks. However highest tendency for heavy TVC is in all OMPB with highest bacteria load in the OMPBC (8.6 × 10^4), 7.6 × 10^4 (OMPBO) and 6.6 × 10^4 (OMPBM). After 12 weeks highest ranged TFC of (0.6 - 0.7) × 10^4 was recorded in *M. rume* as against 0.2 × 10^4 recorded in the initial smoked for all. TCC was highest in *C. gariepinus* (4.0 - 4.3) × 10^4. Packaging did not limit the existence of micro-organisms. Six bacteria species (*Micrococcus* (*acidiophilus*, *luteus*), *Bacillus* (*subtilis*, *cereus*, *aureus*), *Staphylococcus aureus*, *Streptococcus lactis*, *Proteus* (*vulgaricus*, *morganii*), *Pseudomonas aureginosa*) and three fungi species (*Aspergillus* (*niger*, *tamari*), *Rhizopusnigricans*, *fusariumoxysporum*) were represented in all the packages. On the average five bacteria and two fungi species were represented, excepting for OMPBM and OMPBO with six bacteria species.

Keywords: Bacteria; Coliform; Mould Count/Flora; Freshwater Fish Species; Packaging Material

1. Introduction

Bacteria are unicellular microscopic organisms which occur almost everywhere in nature. Up to 1500 species of bacteria have been isolated since bacteria are living things; they acquire a source of food, moisture and suitable temperature to grow, when these conditions are adequate. Bacteria cause spoilage of improperly dried fish by multiplying inside the fish flesh thereby causing putrefaction. Once bacteria spoilage sets in there it is hard to remedy. The result of bacteria attack is off odour and flavor and when pathogenic bacteria are involved, it could result in illness to consumer [1].

The bacteria that most often involved in the spoilage of fish are part of the natural flora of the external slime of fishes and their intestinal content [2]. They lamented that the predominant kinds of bacteria causing spoilage vary with the temperatures at which the fish are held as follows:

- Chilling temperature
  Species of pseudomonas
  Achromebacter and
  Flavobacterium
- Higher temperature
  Genera micrococcus and

Bacillus

- Atmospheric temperature
  Escherichia
  Proteus
  Serratia
  Sarcina and
  Clostridium

Bacteria are unicellular microscopic organisms which occur almost everywhere in nature [1]. Up to 1500 species of bacteria have been isolated since bacteria are living things. They acquire a source of food, moisture and suitable temperature to grow [3], when these conditions are adequate. Bacteria will grow by a process known as Binary Fission in which the cell divides into two new cells. Some bacteria causing fish spoilage might have a generation time of 20 minutes at 30˚C [1]. In such a case, a single bacterium may give billions in 10 hours [4].

Whilst increase in the population of micro-organisms by geometric progression is theoretically possible, its practical application is limited by environment factors prevailing. These factors are:

1) Temperature

Table 1 below shows the ranges of temperature for the growth of micro-organism.

2) Water Content

Table 2 shows the minimum water activity for the growth of micro-organism.

3) Acidity or Alkalinity (pH)

Bacteria grow well over a wide range of hydrogen ion concentration pH ranging from 4.0 - 9.0. The optimum pH growth for most bacteria lies between pH 6.5 and 7.5 although some bacteria are capable of growing at the extremes of the pH ranges. Bacteria growth and toxin production are inhibited if the conditions are more lethal to micro-organisms than alkaline [6].

4) Nutrient Composition

Bacteria are living organisms and like other living things such as plants and animals, they require a source of energy to survive. Such energy can be obtained from sunlight or by breakdown of nutrients which are mainly carbohydrates, proteins, fats and oil, vitamins and other growth factors. The breakdown of each of these nutrients requires the possession of the appropriate enzymes by bacteria [1,6].

Table 1. Temperature ranges for growth of micro-organism.

Types of micro-organism	Minimum (˚C)	Optimum (˚C)	Maximum (˚C)
Psychrophiles	0	15 - 25	30
Mesophiles	10	37	43
Thermopliles	25	50 - 65.5	85

Table 2. Minimum water activity for growth of micro-organism [5].

Micro-organism	Minimum water activity
Normal bacteria	0.90
Normal yeast	0.88
Normal moulds	0.80
Halophilic bacteria	0.75
Dryness resistant moulds	0.05
Osmotic pressure resistant yeast	0.61

1.1. Bacterial in Smoked Fish

Smoked fish and shellfish products can be a source of microbial hazards including *listeria monocytogenes*, Salmonella species and *Clostridium botulinium*, *L. mnonocytogens* has been identified in several food borne outbreaks, in which pasteurized milk, coleslaw and soft cheese were implicated [7]. These organisms have also been isolated from a variety of fish and shellfish products [8].

1.2. Fungal Attacks in Smoked Fish

Insufficient dried fish (still containing approximately 40% moisture) especially at the processing location are prone to fungal infection, principally from the non-specific *Penicillium spp.*, *Aspergillus spp.* Substantial qualities of fish are usually discarded during drying due to fungal growth. Fungal spp. also associated with smoked fish include: *Aspergillus fumigates*, *Absidia spp.*, *Rhizopus spp.*, *Mucor spp.*, *Cladosporium spp.* [9-12]. It was observed that though smoking fish provides longer shelf life than other preservative methods, smoking will be effective if properly done (especially to reduce packaging).

Adebayo-Tayo *et al.* [9] identified 12 different fungi and aflatoxin B1 and G1 in three main markets in Nigeria on smoked dried fish with moisture content ranging from 22.7% - 27.6%. He said the level of infestation might be due to high percentage of moisture content of the smoked fish.

2. Materials and Methods

2.1. Collection of Samples\Packaging

Twenty pieces (sample) of each fish species of average weight 250 grams were collected for *Oreochromisniloticus* (O), *Clariasgariepinus* (C) and *Mormyrusrume* (M). Also fresh samples were collected for the initial proximate analysis while the remaining fresh fishes were transported to the processing unit for smoking. After which the initial proximate analysis of the hot smoked fish was also taken before packaging in the 37 cm × 25 cm packaging materials for each of the smoked fish species (using each of the three different packaging material for each fish species) at the rate of six (6) fish species per package and labeled e.g. for Oreochromis (STPBO—Sealed Transparent Polythene Bag Oreochromis, SPBO—Sealed paper Bag Oreochromis, OMPBO—Open Month Polythene Bag Oreochromis.

2.2. Hot Smoking of the Fish Species

The smoking kiln was locally improvised. Three broken blocks each of 0.3 m height was used to raise the wire gauze (on which the fish were laid) to avoid direct contact with fire. Big wire gauze of mesh size 2 cm was set on the fire when the fire was fully lit. The three species of the fish to be smoked were placed on the gauze. Big aluminum basin with a opening at the centre was used to cover the fish species in order to conserve the fire. It was through the opening that the temperature of the smoking kiln (chimney) was taken daily, until the three fish species were hot smoked dried. Hot smoking was done for 36 hours (this was achieved in three days at an average of 12 hours smoking per day) at an average temperature of 100˚C.

Hot smoking was done with an exotic hard wood (Eucalyptus species), collected from the Forestry Department of the University of Ibadan. Turning of the fish species were done at the same time to maintain uniform drying\smoking at an interval of one hour (1.5 hr) thirty minutes for 3 days.

2.3. Packaging and Shelfing

After three days of intensive smoking, each species of the three freshwater fish species were packaged under three different packaging materials (Sealed Transparent Polythene Bag (STPB), Sealed Paper Bag (SPB) (Brown envelope), Open Mouth Polythene Bag (OMPB) (Black in colour)) under room ambient temperature range of 25˚C - 32˚C for 12 weeks. Mould growth: insect infestation was checked daily during this period for each of the fish species.

The three different materials used were:

A. Sealed Transparent Polythene Bag (STPB)

1. Tilapia (*Oreochromisniloticus*) (STPBO)
2. *Clariasgariepinus* (STPBC)
3. *Mormyrusrume* (STPBM)

B. Sealed Paper Bag (SPB)

1. Tilapia (*Oreochromisniloticus*) (SPBO)
2. *Clariasgariepinus* (STBC)
3. *Mormyrusrume* (STBM)

C. Open Mouth Polythene Bag (OMPB)

1. Tilapia (*Oreochromisniloticus*) (OMPBO)
2. *Clariasgariepinus* (OMPBC)
3. *Mormyrusrume* (OMPBM)

The fishes were packaged hot in the packaging bags and stored in the laboratory for 12 weeks.

2.4. Preparation of Media

All analytical procedures in this study are according to the A.O.A.C [13].

2.4.1. Nutrient Agar

Twenty eight (28) grams of powdered commercially prepared of nutrient agar was weighed on Analytical metller balance into a clean dry 1 litre conical flask and 1000 ml of distilled water placed inside a water bath set about 90˚C, allow the agar to dissolve. Distribute them into MacCantney bottles and placed them inside autoclave and set the autoclave at 121˚C for 15 mins.

2.4.2. Macconkey Agar (Mcca)

Fifty five (55) grams of macConkey Agar was weighted into a 1 litre capacity of conical flask and brings to boil to dissolve the agar. Distribute them into Mac Cartney bottles and autoclave as for Nutrient Agar.

2.4.3. Potato Dextrose Agar (PDA)

Thirty nine (39) grams of PDA was weighted into a 1 litre capacity of conical flask bring to boil and distributed them into Mac Cartney bottles and placed them inside an autoclave as for Nutrient Agar.

2.5. Pouring of Plates

After autoclaving the media were placed inside a water bath set at 45˚C to maintain the media in a molten state.

1 g each of the sample was weighed into a test-tube containing 9 ml of sterile distilled water and serially dilute them until you reach your dilution factor (10-5) and plate out 1 ml of the last dilution factor into a sterile plates (sterilized by placing them in an over set at 160˚C for an hour). Pour the media individually *i.e.* Nutrient Agar, Mac Conkey Agar and Potato Dextrose Agar into a separate plate *i.e.* each sample will have 3 plates and they were duplicated.

After solidifying the plates were incubated in an incubator set at 370˚C for Nutrient Agar and Mac Conkey Agar while the potato Dextrose Agar was incubated at 280˚C - 30˚C. All the plates were incubated invertedly.

2.6. Microbial Count

The plate was counted at 48 hours for Nutrient Agar and Mac Conkey Agar while it was read for potato Detrose Agar t 72 hours.

2.7. Lactic Acid Bacterial Count

Fifty five (55) grams of Man De Rogsa and shape medium (MRS) was weighed as for the above nutrient agar preparation procedures.

2.8. Statistical Analysis

Analysis of Variance (ANOVA) in completely randomized design was performed on the data obtained using SPSS (2006). Significant means were compared at 5% probability level using Duncan's New Multiple Range Test (DMRT) as provided in the same SPSS (2006).

3. Result

As shown in **Table 3** the microbial load varied significantly ($P < 0.05$) among the three species. In the fresh fish the highest TVC of 12.4×10^4 was recorded in *C. gariepinus*, this is followed by *O. niloticus* with 10.6×10^4 and lastly *M. rume* 9.8×10^4. However while TFC was zero in the fresh fish for the three fish species, highest TCC of 0.8×10^4, was recorded for *O. niloticus*, followed by *C. gariepinus* (0.4×10^4) and lastly *M. rume* with TCC of 0.3×10^4.

Table 4 and **Figure 1** show that the TVC of *O. niloticus* dropped from 10.6×10^4 (in the fresh state) to 8.4×10^4 in the initial hot smoked and *M. rume* dropped from 9.8×10^4 (fresh state) to 7.0×10^4 after hot smoking, while the TVC of *C. gariepinus* slightly increased from 12.4×10^4 in the fresh state to 12.6×10^4 after hot smoking. While TFC increased from zero to 0.2×10^4 for the three fish species; highest TCC of 9.8×10^4 was recorded in *C. gariepinus*, followed by 4.2×10^4 in *O. niloticus* and lastly 3.0×10^4 in *M. rume*.

Table 5 shows that the least bacteria load (TVC) was recorded in the SPBM and STPBM, both recording TVC 6.4×10^4 in each case. Generally highest TVC of 8.6×10^4 (OMPBC), 8.3×10^4 (SPBC) and 8.2×10^4 (STPBC) were recorded in all *C. gariepinus* among the nine packages at the end of 12 weeks storage/packaging. Next is *O. niloticus* packaging 7.6×10^4 (OMPBO), 7.4×10^4 (STPBO) and 7.2×10^4 (SPBO) and lastly *M. rume* $6.6 \times$

Table 3. Microbial load of fresh fish samples.

Fish species	Total Viable Count (TVC)	Total Coliform Count (TCC)	Total Fungi Count (TFC)
C. gariepinus	12.4×10^4	0.4×10^4	NIL
O. niloticus	10.6×10^4	0.8×10^4	NIL
M. rume	9.8×10^4	0.3×10^4	NIL

Table 4. Microbial load of initial hot smoked fish.

Fish species	Total Viable Count (TVC)	Total Coliform Count (TCC)	Total Fungi Count (TFC)
C. gariepinus	12.6×10^4	9.8×10^4	0.2×10^4
O. niloticus	8.4×10^4	4.2×10^4	0.2×10^4
M. rume	7.0×10^4	3.0 × 104	0.2×10^4

Table 5. Final microbial load at the end of twelve weeks storage/packaging of the three hot smoked freshwater fish species.

Fish species	Total Viable Count (TVC)	Total Coliform Count (TCC)	Total Fungi Count (TFC)
SPBC	8.3×10^4	4.0×10^4	0.6×10^4
OMPBC	8.6×10^4	4.3×10^4	0.6×10^4
STPBC	8.2×10^4	4.2×10^4	0.5×10^4
SPBO	7.2×10^4	3.8×10^4	0.4×10^4
OMPBO	7.6×10^4	3.4×10^4	0.3×10^4
STPBO	7.4×10^4	3.3×10^4	0.5×10^4
SPBM	6.4×10^4	3.2×10^4	0.6×10^4
OMPBM	6.6×10^4	3.1×10^4	0.7×10^4
STPBM	6.4×10^4	3.2×10^4	0.7×10^4

10^4 (OMPBO), 6.4×10^4 (SPBM) and 6.4×10^4 (STPBM) respectively. However all the OMPB packages (Open Mouth Polythene Bag)—OMPBC (8.6×10^4), OMPBO (7.6×10^4) and OMPBM (6.6×10^4) had the highest bacteria load in each of the 3 fish species. However the highest ranged fungi (TFC) of 0.6×10^4 - 0.7×10^4 was recorded in *M. rume*. This is followed by *C. gariepinus* with 0.5×10^4 - 0.6×10^4 while least TFC range of 0.3×10^4 - 0.5×10^4 was recorded in *O. niloticus*. TCC was highest in *C. gariepinus* ranging from 4.0×10^4 - 4.3×10^4 followed by *O. niloticus* (3.3×10^4 - 3.8×10^4) and lastly *M. rume* (3.1×10^4 - 3.2×10^4) respectively.

Generally *M. rume* was the best packaged in terms of bacteria load (TVC) with the least range of (6.4×10^4 - 6.6×10^4) followed by *O. niloticus* (7.2×10^4 -7.6×10^4) and lastly *C. gariepinus* (8.2×10^4 - 8.6×10^4) which is the poorest in terms of bacteria loads. There were significant ($P < 0.05$) differences between and within the TVC (*i.e* bacteria load), TCC and TFC for the three species in this study.

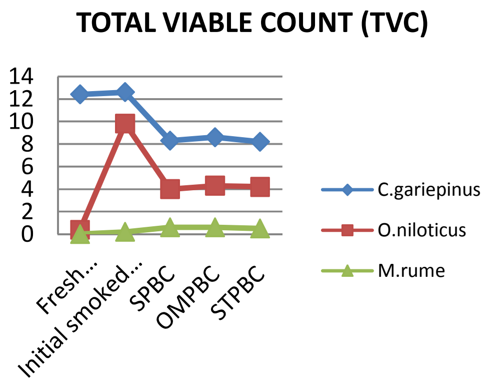

Figure 1. Total Viable Count (TVC) (Bacteria load) for the three fish species for the fresh fish, initial hot smoked and final/smoked packaged at the end of 12 weeks.

Table 6 shows that 6 bacteria species were identified in the fresh *O. niloticus*, while 5 species each were identified for *C. gariepinus* and *M. rume* in their fresh state. Also *Micrococcus acidiophilus* and *Proteus vulgaricus* were identified in the three fresh fish species under study, while *Streptococcus lactis* and *Staphylococcus aureus* were absent in *C. gariepinus*. However *Serraticmacescenes* was only present in the fresh *C. gariepinus*.

Table 7 shows that only *C. gariepinus* had only one fungi species (*Rhizopusnigrica*) represented in the initially hot smoked three (3) fish species. While 6 bacteria species were each represented in *C. gariepinus* and *M. rume*; *O. niloticus* had 5 bacteria species; also only *Staphylococcus aureus* was present throughout in the 3 initially hot smoked fish species.

Table 8 shows that only the OMPB for *M. rume* had 6 bacteria species and 2 fungi species, while the remaining 8 packages had 5 bacteria species and 2 fungi species. The prominent fungi species represented all the 9 packages are *Aspergillu ssp* (*niger*, *tamari*), *Rhizopusnigricans* (in SPBO, OMPBO, OMPBC, SPBM AND STPBM), WHILE *Fusariumoxysporum* is only represented in STPBO.

The prominent bacteria species represented in all the 9 packages are *Micrococcus sp* (*acidiophilus and luteus*), *Bacillus sp* (*aureus*, *cereus* and*luteus*). *Staphylococcus aureus* is present in 8 packages with the exception of OMPBO, *Streptococcus lactis* is also present in 8 packages excepting SPBO. *Proteus sp* (*vulgaricus* and *morgani*) were presented in 7 packages, excepting OMPBC and STPBM. Lastly, *Pseudomonas aureginosa* is present in only 3 packages (SPBO, OMPBO and OMPBC). Since micro-organisms are ubiquitous the type of pack aging (as shown in the study) will not limit their existence.

Tables 6. Bacteria species identified from the fresh three fish species.

Fish species	Micro organism
C. gariepinus	*Micrococcus acidiophilus*, *Bacillus cereus*, *Serraticmacescenes*, *Bacillus subtilis*, *Proteus vulgaricus* (5 bacteria species)
O. niloticus	*Pseudomonas auregionosa*, *Streptococcus lactis*, *Micrococcus acidiophilus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Proteusvulgaricus* (6 bacteria species)
M. rume	*Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus acidiophilus*, *Proteusvulgaricus*, *Streptococcus lactis* (5 bacteria species)

Table 7. Bacteria and fungi species identified from the initial hotsmoked three fish species.

Fish species	Micro organism
C. gariepinus	*Micrococcus luteus*, *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus lactis*, *Pseudomonas aureginosa*, *Proteus vulgaricus*, 6 bacteria species + 1 fungi (*Rhizopusnigrica*)
O. niloticus	*Streptococcus lactis*, *Micrococcus acidiophilus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus acidiophilus*, *Bacillus macerans.* 5 bacteria species + Nil (0) fungi species
M. rume	*Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus acidiophilus*, *Micrococcus luteus*, *Proteus morganii*, *Pseudomonas aureginosa* (6 bacteria species + Nil (0) fungi species)

Table 8. Bacteria and fungi species identified from the smoked three fish species at 12 weeks of storage/packaging.

Packaging	Micro-organism (bacteria and fungi species)
SPBC	*Bacillus cereus*, *Streptococcus lactis*, *Staphylococcus aureus*, *Proteus vulgaricus*, *Micrococcus acidiophilus*, 5 bacteria + 2 fungi species *Aspergillustamari*, *Aspergillusniger*
OMPBC	*Staphylococcus aureus*, *Bacillus cereus*, *Micrococcus luteus*, *Pseudomonas aureginosa*, *Streptococcus lactis*, 5 bacteria + 2 fungi species *Rhizopusnigricans*, *Aspergillusniger*
STPBC	*Staphylococcus aureus*, *Bacillus cereus*, *Micrococcus acidiophilus*, *Proteus vulgaricus*, *Streptococcus lactis*, 5 bacteria + 2 fungi species *Aspergillustamari*, *Aspergillusniger*
SPBO	*Micrococcus luteus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus vulgaricus*, *Pseudomonas aureginosa*, 5 bacteria + 2 fungi species *Rhizopusnigricans*, *Aspergillusniger*
OMPBO	*Streptococcus lactis*, *Micrococcus acidiophilus*, *Bacillus cereus*, *Streptococcus lactis*, *Proteus vulgaricus*, *Pseudomonas aureginosa*, 6 bacteria + 2 fungi species *Rhizopusnigricans*, *Aspergillusniger*
STPBO	*Micrococcus acidiophilus*, *Streptococcus lactis*, *Proteus vulgaricus*, *Bacillus cereus*, *Staphylococcus aureus*, 5 bacteria + 2 fungi species *Aspergillusniger*, *Fusariumoxysporum*
SPBM	*Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus macerans*, *Streptococcus lactis*, *Proteusmorganii*, 5 bacteria + 2 fungi species *Rhizopusnigricans*, *Aspergillustamari*
OMPBM	*Staphylococcus aureus*, *Bacillus cereus*, *Proteus vulgaricus*, *Bacillus subtilis*, *Streptococcus lactis*, *Micrococcus acidiophilus*, 6 bacteria + 2 fungi species *Aspergillustamari*, *Fusariumoxysporum*
STPBM	*Micrococcus acidiophilus*, *Micrococcus leteus*, *Streptococcus lactis*, *Staphylococcus aureus*, *Bacillus subtilis*, 5 *bacteria* + 2 *fungi species Rhizopusnigricans*, *Aspergillusniger*

4. Discussion

The highest bacteria load (TVC) of 12.4 × 10^4 was recorded in the fresh *C. gariepinus* followed by 10.6 × 10^4 in *O. niloticus* and lastly 9.8 × 10^4 in the fresh *M. rume*. However, initial hot smoked reduced the bacteria load of *O. niloticus* to 8.4 × 10^4 and *M. rume* to 7.0 × 10^4 while the initial hot smoked *C. gariepinus* TVC of 12.6 × 10^4 was not affected by hot-smoking since a slight increase of 0.2 × 10^4 was recorded after hot smoking.

Packaging had a significant ($P < 0.05$) effect at 12 weeks storage/packaging of smoked fish for *C. gariepinus* which reduced from 12.6 × 10^4 TVC to OMPBC (8.6 × 10^4)—SPBC (8.3 × 10^4)—STPBC (8.2 × 10^4) and *M. rume* with TVC reducing from 7.0 × 10^4 (in the initial hot smoked fish) to 6.6 × 10^4 (OMPBM)—6.4 × 10^4 (SPBM)—6.4 × 10^4 (STPBM). However all the OMPB packages (Open Mouth Polythene Bag)—OMPBC (8.6 × 10^4), OMPBO (7.6 × 10^4) and OMPBM (6.6 × 10^4) had the highest bacteria load in each of the 3 fish species. This is also revealed in **Figure 1**.

Total Coliform Count (TCC) generally increased from the fresh fish sample 0.4 × 10^4 to 9.8 × 10^4 (*C. gariepinus*) 0.8 × 10^4 to 4.2 × 10^4 (*O. niloticus*) and 0.3 × 10^4 to 3.0 × 10^4 in the initial smoked (*M. rume*). Total Coliform Count (TCC) dropped significantly ($P < 0.05$) from 9.8 × 10^4 in the initial hot smoked *C. gariepinus* to a range of 4.0 × 10^4 - 4.3 × 10^4 in all the 3 *C. gariepinus* packaging, while TCC virtually remained the same for the *M. rume* packaging and dropped from 4.2 × 10^4 to a range of 3.3 × 10^4 - 3.8 × 10^4 for *O. niloticus* at the end of 12 weeks. This is shown in **Figure 2**.

No Fungi count was recorded in the fresh fish sample for the 3 fish species. However a value of 0.2 × 10^4 fungi count was recorded for the 3 fish species after initial hot smoking. This value increased; highest for *M. rume* (0.6 × 10^4 to 0.7 × 10^4) next is 0.4 × 10^4 to 0.6 × 10^4 in *C. gariepinus* and lastly 0.3 × 10^4 - 0.5 × 10^4 in *O. niloticus*. Since micro-organisms are ubiquitous the type of packaging (as shown in this study and **Figure 3**) will not limit their existence.

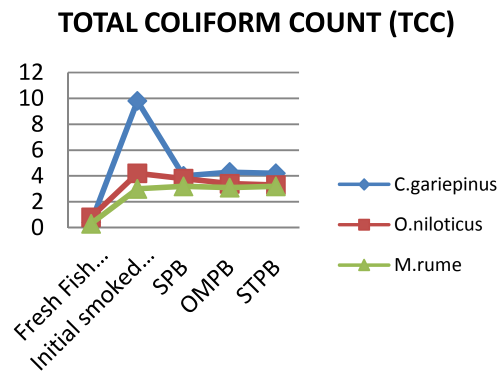

Figure 2. Total Coliform Count (TCC) for the three fish species for the fresh fish, initial hot smoked and final/smoked packaged at the end of 12 weeks.

The bacterial load (TVC) count for all the three species of fish in the nine packages used for this study are below the maximum bacteria count of 5 × 10^5 cfu for good fish product according to the International Commission on Microbiology Safety for Food [14].

For *C. gariepinus* significant ($P < 0.05$) decreases were observed in the TCC 9.8×10^4 in the initial smoked fish which reduced to a range of 3.1 × 10^4 - 3.2 × 10^4 at the end of 12 weeks. This was in conformity with Wil-

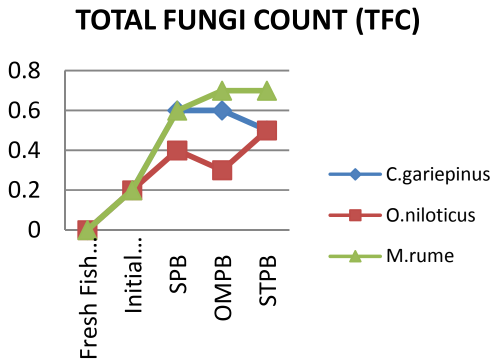

Figure 3. Total Fungi Count (TFC) for the three fish species for the fresh fish, initial hot smoked and final/smoked packaged at the end of 12 weeks.

liam, C.F and Dennis, C.W [15] who reported that the faecal coliforms count of fresh *C. gariepinus* fillets were similarly low after 8 days of cold storage.

Table 3 shows that there was absence of fungi in the fresh sample of the three fish species, while in **Table 4** only one species of fungi (*Rhizopusnigrica*) was present in the initially smoked *C. gariepinus*. At the end of the 12 weeks of storage/packaging three (3) more fungi species (*Aspergillusniger*, *Aspergillustamari* and *Fusarumoxysporum*) were represented at the rate of 2 fungi species per packaging. That is fungi species were represented in all the 9 packages. The results obtained were similar to those observed by Adebayo-Tayo *et al.* and Fafioye, O.O *et al.* [9,16]. During storage of smoked fish product there was significant ($P < 0.05$) increase in the fungi count with length of storage as seen in this study. This is in line with Oyebamiji, O. F *et al.* and Wogu, M.D *et al* [11,12] who worked on stored smoked fish products marketed in the open market. The presence of fungi may be due to the difference in the chemical composition of the fish species and to which different moulds react differently [16,17].

Only the OMPBM and OMPBO had 6 bacteria species represented while the remaining 7 packages had 5 bacteria species. The prominent bacteria species represented in all the nine (9) packages are *Micrococcus* sp (*acidiophilus and luteus*), *Bacillus* sp (*aureus*, *cereus and luteus*), *staphylococcus aureus* (is present in 8 packages) except in OMPBO. *Streptococcus lactis* also in 8 packages excepting SPBO. Others are *Proteus vulgaricus*, *P. morganii* and *Pseudomonas aureginosa*.

5. Conclusion

Highest Bacteria Count (TVC) was recorded in *C. gariepinus* packages among the nine packages at the end of 12 weeks. The 3 packaged fishes for *C. gariepinus* had the highest bacteria load with OMPBC (Open Mouth Polythene Bag Being the Highest). Highest tendency for heavy bacteria load (TVC) is in the Open Mouth Polythene Bag which has been confirmed in the OMPB for all the 3 fish species. Highest ranged Total Fungi Count (TFC) was recorded in *M. rume* followed by *O. niloticus*. Total Coliform Count (TCC) was highest in *C. gariepinus* followed by *O. niloticus*. Packaging did not limit the existence of micro-organisms. There were 5 bacteria species and 2 fungi species represented in each of the packages (with the exception of OMPBM and OMPBO with 6 bacteria species). The prominent fungi species represented in the 9 packages at the end of 12 weeks are *Aspergillus* species (*niger* and *tamari*), *Rhizopusnigricans* and *Fusariumoxysporum*. Prominent bacteria species represented in all 9 packages are *Micrococcus* species (*acidiophilus* and *luteus*), *Bacillus* species (*aureus*, *cereus* and *luteus*). *Staphylococcus aureus* is present in 8 packages (excepting OMPBO) and also *Streptococcus lactis* (excepting SPBO). *Proteus* species (*vulgaricus and morganii*) (in 7 packages excepting OMPBC and STPBM) and lastly *Pseudomonas aureginosa* are present in only 3 packages (SPBO, OMPBO and OMPBC).

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Abbreviations

TVC: Total Viable Count
TCC: Total Coliform Count
TFC: Total Fungi Count
SPBC: Sealed Paper Bag-*Clariasgariepinus*
OMPBC: Open Mouth Polythene Bag-*Clariasgariepinus*
STPBC: Sealed Transparent Polythene Bag-*Clariasgariepinus*
SPBO: Sealed Paper Bag-Tilapia (*Oreochromisniloticus*)
OMPBO: Open Mouth Polythene Bag-Tilapia (*Oreochromisniloticus*)
STPBO: Sealed Transparent Polythene Bag-Tilapia (*Oreochromisniloticus*)
SPBM: Sealed Paper Bag-*Mormyrusrume*
OMPBM: Open Mouth Polythene Bag-*Mormyrusrume*
STPBM: Sealed Transparent Polythene Bag-*Mormyrusrume*