EFFECT OF SELECTED OIL TREATMENT ON MICROBIAL LOADS OF SOME SMOKED FRESHWATER FISH SPECIES

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ABSTRACT
The study investigated the effect of selected oil treatment on microbial load of some smoked freshwater fish species. Forty-two (42) fish samples comprising Clarias gariepinus, Heterotis niloticus and Proptopterus annectens with mean weight of 650g was procured from Wadata fish landing site, Makurdi, Benue State, Nigeria. The fish samples were washed and grouped into batches, immersed in 5 % brine solution for 30 minutes within ambient temperature of 30±2 ^{0}C. They were smoked using smoking kiln. Batch A, (Control) was not treated while the other three batches- B, C and D were immersed in 30 ml of soybean, groundnut and castor oil respectively and dried on laboratory table. Subscripts 1, 2 and 3 indicate species from C. gariepinus, H. niloticus and P. annectens respectively. Smoked fish samples were subjected to microbial analysis. The results indicated that oil application, fish species and storage duration are significant ($p<0.05$) effect on microbial load. The total viable count (TVC) ranged from 4.01 to 5.17 LogCFU/g as storage duration increases. Total coliform count (TCC) was least (3.95±0.11 LogCFU/g) in C. gariepinus and greatest in P. annectens. Mean values of TCC increased with storage time from 3.38± 0.07 LogCFU/g at week 0 to 4.50±0.05 LogCFU/g. Total fungal count (TFC) increased from 3.44 ± 0.03 to 4.12±0.03 LogCFU/g as storage time increased from 0 to 8 weeks. Groundnut, soybean and castor oil have demonstrated effect in controlling microbial count below control level. Castor oil showed the least potential of keeping lowest microbial counts after the duration of 8 weeks.

Keywords: Smoked fish, Storage duration, Oil application, Microbial analysis, Microbial count.

INTRODUCTION

Fish is one of the cheapest sources of animal protein and other essential nutrients required in human diets. Fish is becoming increasingly important in the diet of a larger percentage of the populace worldwide because of its availability, palatability and health provisions (Idris *et al*., 2010). With their high protein content, fish are a natural supplement in human diet. However, the demand for high quality fish and fishery products is growing significantly every year mostly due to their nutritional fact that they contain much beneficial healthy substances (Pal *et al*., 2018). As important as fish is, high degree of fish spoilage still occur in Nigeria and serves a major constraint to the development of fishing industry in Nigeria (Akinpelu *et al*., 2013). Blackwell (2014) characterized fish as a very perishable product and processing is therefore necessary to assure safety and prolonged shelf life of fish. fish are a very perishable commodity, more than cattle, sheep, and poultry, and get spoiled very easily even in temperate climates. Traditional processing methods of fish including smoking were originally developed to preserve fisheries products by lowering the water activity which prevents the growth of spoilage bacteria. Hence the need for more proper processing method to ensure they meet the requirement of food regulatory bodies and commercial specifications. (Kose, 2010). *Clarias gariepinus*, is a very fresh water fish in Nigeria as it enjoys wide acceptability in most parts of the country because of its unique taste, flavour and texture (Ayeloja *et aI*., 2011). *Heterotis niloticus* is another commercial fish is a highly preferred source of food because of its high protein content and hardy flesh, thus forming a very important component in the diet of many Nigerians (Adam and Suleiman, 2021). *Protopterus annectens* also called African lungfish is widely distributed in Africa and generally inhabits shallow waters such as swamp and marshes. Its meat has a characteristic strong taste and is rich in protein, potassium and phosphorus (llozumba, and Ezeife, 2009). One effective method for the extension of shelf life of smoked fish has proven to be the use of Vegetable oil which are aromatic oily liquids obtained from plants buds, seeds, and nuts (Lee, *et al* 2007). This oil can be used in food in order to prolong the shelf life due to of the natural antioxidant and antimicrobial properties, and additionally, they can reduce or replace synthetics additives plant material (Erkan, *et al.* 2012). Some studies have shown that some vegetable oils have marked antimicrobial, antibacterial, antiparasitic, antiviral, antimycotic, and antioxidant activities in smoked fish (Sacchetti, *et al* 2005).

Groundnut oil is the organic oil derived from peanuts. (Merck, 2015). The Soybean oil is used as cooking oil, and in a wide range of application in processed foods (Merck, 2015). Castor oil is a vegetable oil obtained from castor oil plant. (Patel *et al*., 2016). The oil is not only used as a flavouring agent, but also as a preservative, due to its

antioxidant, antifungal and antibacterial activity (Matthews, 2010). In Nigeria, fish merchants rub groundnut and other vegetable oils on dried fish but only little information is available on whether such practices are protective or merely cosmetic. The study therefore examined the effect of Groundnut, Castor and Soybean oil on microbial load of smoked *Clarias gariepinus, Heterotis niloticus* and *Protopterus annecterns* stored under ambient temperature.

MATERIALS AND METHODS

Study Area

The study was conducted at the Department of Fisheries and Aquaculture, Joseph Sarwuan Tarka University, Makurdi, Benue State, Nigeria, located on longitude 7.3 ^{0}N and latitude 8.54 ^{0}E.

Procurement and preparation of Samples

Fresh fish samples ((*Clarias gariepinus, Heterotis niloticus and Protopterus annectens*)) were purchased from fishermen at fish landing site, Wadata, Makurdi, Benue State. Groundnut, soybean and castor was obtained from Wadata market in Makurdi Benue state. The fish were transported in an insulated container. The length and weight of the fish samples were taken using a measuring tape and sensitive scale (Atom A 122 Electronic kitchen digital weighing scale, model SF: 400A). The fish was scaled, eviscerated and washed before allowing to drain for 40 minutes. The drained fish samples were skewered and smoked-dried using charcoal drum smoking kiln.

Experimental Design

Each of the three smoked fish species was divided into four batches (A B C and D) for the control and treatments. The 3 fish species tagged subscripts 1,2 and 3. Subscripts 1 (*Clarias gariepinus*) was divided into samples A_1, B_1, C_1, and D_1. The Subscripts 2 *(Heterotis niloticus*) was divided into samples A_2, B_2, C_2 and D_2. Subscripts 3 (*Protopterus annectens*) was divided into sample A_3, B_3, C_3 and D_3. The fish samples in Batch A were not treated with oil while the other three batches B, C and D were immersed in 30 mL of groundnut, soybean and castor oil respectively after smoking, dried on laboratory table and stored for 8 weeks under ambient temperature.

Microbial analysis

For evaluating the influence of oils application on the microbiological stability of fish products, several microbiological indicators were assessed: Smoked *C. gariepinus, H. niloticus* and *P. annectens* treated with ground nut, soybean and castor oils were assessed for total viable count (TVC), total coliform count (TCC) and total fungal count (TFC) before and after 4 and 8 weeks of storage.

Total viable count (TVC

Total viable count (TVC) of smoked fish was carried out in triplicate using the method of ICMSF (2002) 1.0 g sample of the smoked fish was weighed using Methler Toledo Weighing Balance and Homogenized by blending to ensure accurate results. Then serial dilution of the homogenized sample was done using sterile saline solution as a suitable diluent. Plate Count Agar (PCA) was selected and plates were incubated at appropriate 37^o C for 48 hours for the growth of the microorganisms. After incubation, the number of colonies on plates containing 30-300 colonies was counted (for optimal accuracy).

TVC was calculated using the formula:

$$TVC = (Number\ of\ Colonies) \times (Dilution\ Factor) \times (Inverse\ of\ Sample\ Volume)$$

Results was expressed as colony-forming units per gram (CFU/g) of the original sample.

Total coliform count (TCC)

Total coliform count (TCC) was determined according to the method of ICMSF (2002). The determining the total coliform count of smoked fish involves a similar process to the total viable count but specifically targets coliform bacteria, which serve as indicators of fecal contamination and general hygiene in food products. Here, aliquots of each dilution was plated onto MacConkey Agar and incubated for 48 hours at 35-37°C. After incubation, the number of typical coliform colonies (pink to red in colour) on the plates were counted. Total coliform count was calculated using the formula:

$$Total\ Coliform\ Count = (Number\ of\ Coliform\ Colonies) \times (Dilution\ Factor) \times (Inverse\ of\ Sample\ Volume)$$

Result was expressed as colony-forming units per gram (CFU/g) of the original sample.

Total fungal count (TFC)

Total fungal count (TFC) was assessed using the method of ISO (2008). Five grams (5.0 g) of smoked fish sample was aseptically collected using sterile tools to prevent contamination and placed in a sterile container after which it was homogenized by blending using an electrical blender to ensure uniform distribution of the sample. Serial dilution of

the sample was prepared using sterile solution and appropriate volumes of the homogenized sample was transferred into dilution tubes and labelled accurately. Fungal colonies were manually counted on each Petri dish after incubation period and the number of colonies were recorded for each dilution.

Total fungal count was calculated per gram of original smoked fish sample using the formula:

$$Total\ fungal\ count\ \frac{CFU}{g} = \frac{Total\ number\ of\ colonies\ counted}{Weight\ of\ sample\ (g) \times Dilution\ factor}$$

Statistical Analysis

In the analysis of the data, an ANOVA via a general linear model was employed, incorporating terms such as species, duration of storage, and types of oil applied as key factors. To discern significant differences among groups, means were separated using Tukey's Honestly Significant Difference (HSD) test, a reliable method for post-hoc analysis. The statistical software Minitab version 21 was utilized for these computations, ensuring accurate and comprehensive results. Significant interactions among the variables were visualized and plotted accordingly, offering a clear depiction of the relationships and patterns within the dataset.

RESULTS

Microbial Load on some Smoked Freshwater Fish Species Treated with selected Vegetable oils and Stored for Eight Weeks

Table1 shows the microbial load on some smoked fish species treated with various vegetable oils and stored for eight weeks. The shows the interaction between the smoked fish species, oil applied and storage duration. The highest (4.79 ± 0.12 LogCFU/g)), (4.79 ± 0.16 LogCFU/g)) and (4.79 ± 0.16 LogCFU/g)) Mean total viable count (TVC) was recorded for *C. gariepinus*, the control and samples stored for 8 weeks respectively. While the lowest (4.54 ± 0.14 LogCFU/g), (4.52 ± 0.15 LogCFU/g) and (4.01 ± 0.07 LogCFU/g) TVC was recorded for *H. niloticus*, CSO treated, and samples stored for week 0 respectively. Mean Total coliform count (TCC) was highest (4.09 ± 0.12 LogCFU/g), (4.28 ± 0.11 LogCFU/g) and (4.50 ± 0.05 LogCFU/g) for *P. annectens*, control and sample storage at week 8 respectively. The lowest (3.95 ± 0.11 LogCFU/g), (3.86 ± 0.11 LogCFU/g) and (3.38 ± 0.07 LogCFU/g) TCC was recoded for *C. gariepinus*, CSO and samples stored for week 0. The value of total fungal count (TFC) recorded the highest (3.83 ± 0.08 LogCFU/g), (3.97 ± 0.07 LogCFU/g) and (3.86 ± 0.05 LogCFU/g) for *C. gariepinus*, control and sample stored for week 4. The lowest TFC was however recorded for *H. niloticus*, CSO treated samples and samples at week 8 storage duration. Among the smoked fish samples assessed, significant (p=0.000) difference exist for the smoked fish species in the TVC TCC and TFC showed significant (p=0.000) difference for samples oil applied while all microbial parameters assessed in the present study showed significant (p=0.000) difference for the storage duration.

Table 1 Microbial Load on some Smoked Freshwater Fish Species Treated with selected Vegetable oils and Stored for Eight Weeks

Treatments	TVC	TCC	TFC
Species		Log.CFU/g	
C. gariepinus	4.79 ± 0.12[a]	3.95 ± 0.11	3.83 ± 0.08
H. niloticus	4.78 ± 0.12[a]	4.04 ± 0.12	3.77 ± 0.06
P. annectens	4.54 ± 0.14[b]	4.09 ± 0.12	3.82 ± 0.07
p-value	0.032	0.166	0.309
Oil Applied			
Control	4.79 ± 0.16	4.28 ± 0.11[a]	3.97 ± 0.07[a]
CSO	4.52 ± 0.15	3.86 ± 0.11[b]	3.69 ± 0.08[b]
GNO	4.72 ± 0.17	4.01 ± 0.14[b]	3.74 ± 0.07[b]
SBO	4.78 ± 0.12	3.95 ± 0.16[b]	3.82 ± 0.09[b]
p-value	0.087	0.000	0.000
Storage Duration			
Week 0	4.01 ± 0.07[c]	3.38 ± 0.07[c]	3.44 ± 0.03[c]
Week 4	4.93 ± 0.11[b]	4.20 ± 0.07[b]	3.86 ± 0.05[b]

Week 8	5.17 ± 0.04[a]	4.50 ± 0.05[a]	4.12 ± 0.03[a]
p-value	0.000	0.000	0.000
Interaction			
Species*Oil*Week	0.000	0.001	0.000

Means in the same column of treatments followed by different superscripts differ significantly ($p<0.05$); interactions differ significantly at $p<0.05$.

TVC= Total Viable Count, TCC =Total Coliform Count, TFC= Total Fungal Count. CSO= Castor oil, GNO= groundnut oil, SBO= Soybean oil

The fit Genera linear model (GLM) results for total viable count (TVC) indicate that the interaction between the oil applied, species of fish and weeks of storage is significant ($p=0.000$). In Figure 1, oil application is associated with a decrease in TVC below that of the controls for *H. niloticus* and *C. gariepinus*. However, oil application caused an increase in TVC above the control level in *P. annectens*. Figure 2 showed a general increase in TVC for all fish species as storage duration increase from week 0 to 8. Total Viable Count (TVC) recorded the least value 3.90 Log.CFU/g for control *P. annectens* at week 0 and the highest value of 5.10 Log.CFU/g). The lowest TVC of 4.02 Log.CFU/g was recorded for smoked *H. niloticus* at week 0 and 5.5Log.CFU/g at week 8.

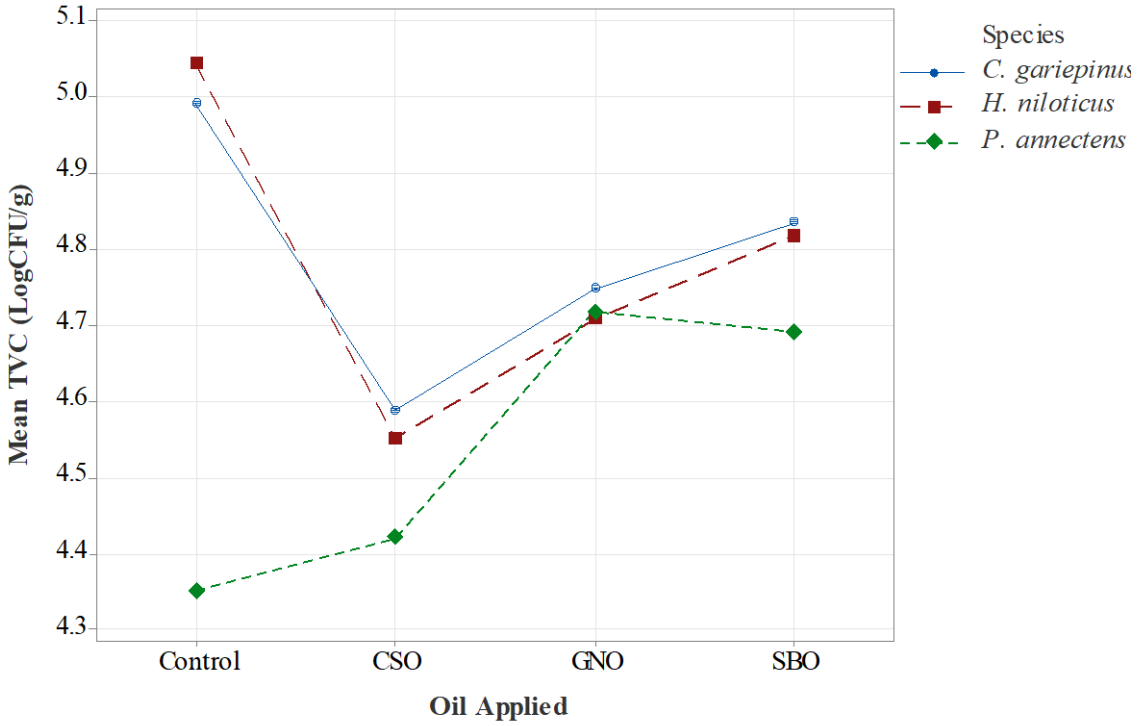

Figure 1; Interaction plot of species versus TVC as determined by oil treatment

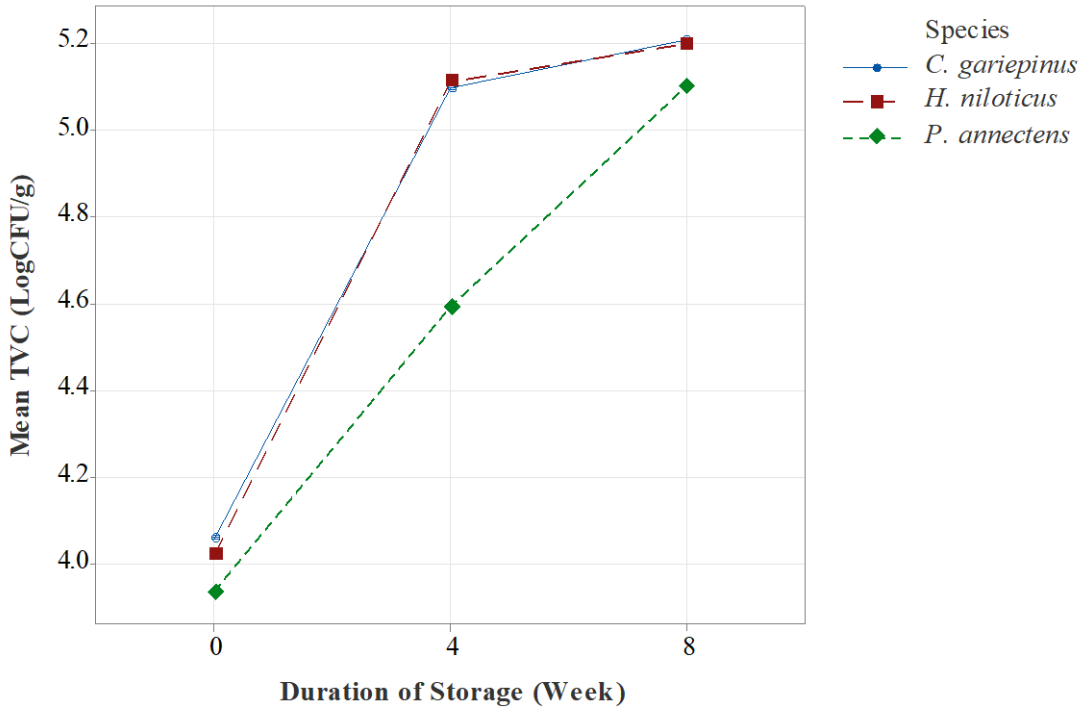

Figure 2: Interaction plot of species versus TVC as determined by the duration of storage

The fit GLM results for TCC indicate that the interaction between the oil applied, species of fish and weeks of storage is significant (p=0.000). In Figure 3 and 4. Oil application is associated with a decrease in TCC below control levels for all the fish species. Castor oil (CSO) is associated with the lowest level of TCC in *C. gariepinus* (3.82 LogCFU/g) while the least levels of TCC associated with GNO and SBO are 38.87 LogCFU (*C. gariepinus*) and 38.87 LogCFU (in *C. gariepinus*) respectively. Among the species applied with oil, GNO treated smoked *H. niloticus* recorded the highest value (4.11 LogCFU/g). interaction plot of species versus TCC as determined by the storage duration in Figure 4

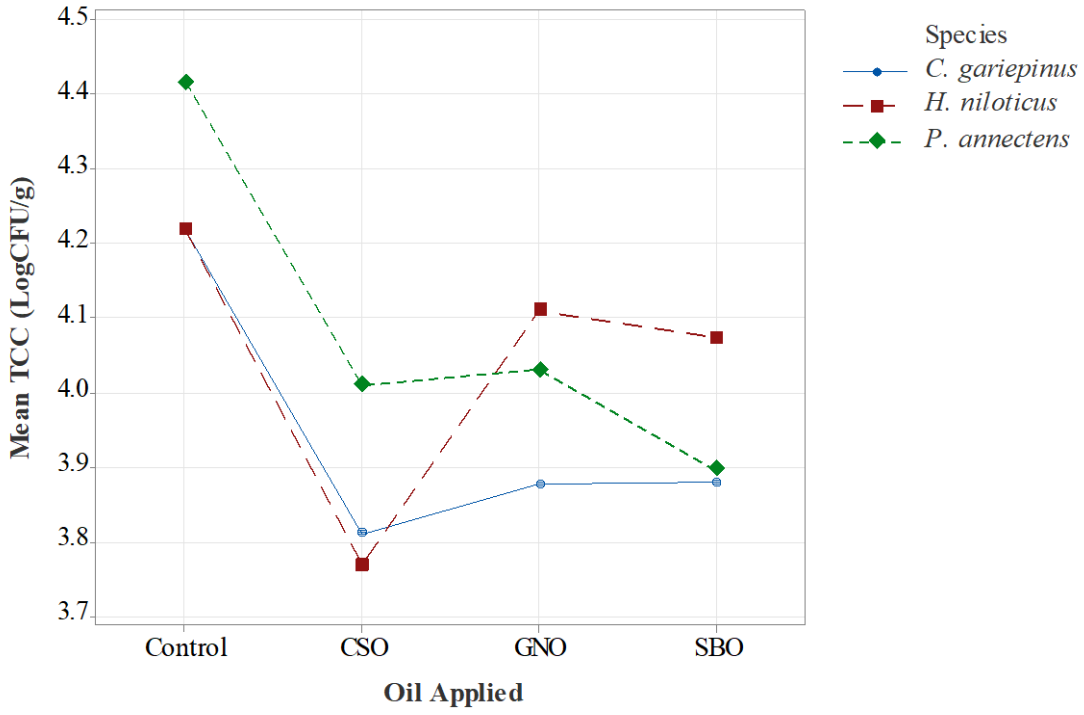

Figure 3: Interaction plot of species versus TCC as determined by oil treatment

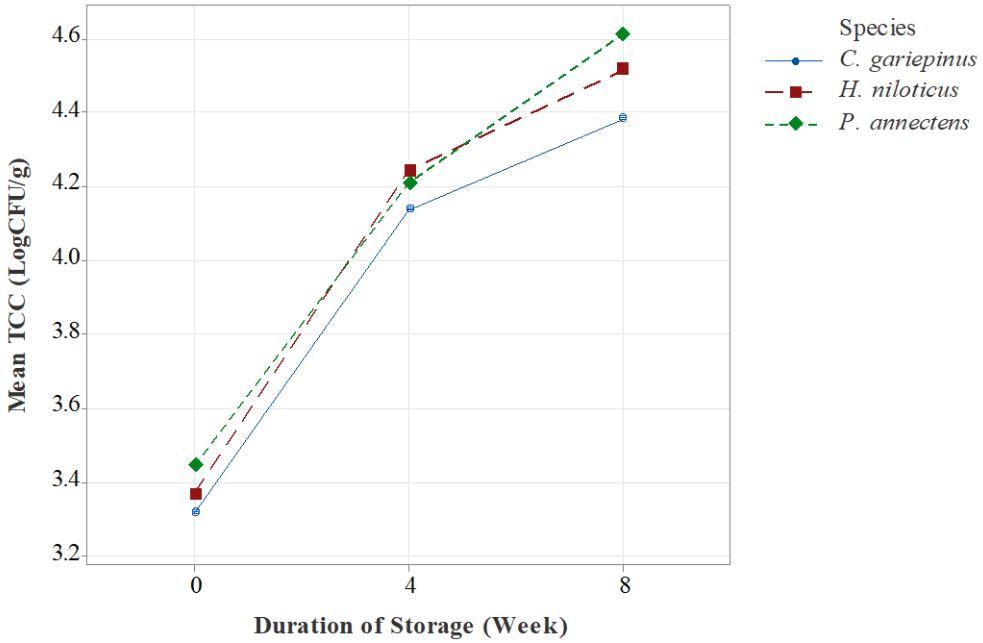

Figure 4: Interaction plot of species versus TCC as determined by the duration of storage

The fit GLM results for TFC indicated that the interaction between the oil applied, species of fish and weeks of storage is significant (p=0.000). In Figure 5, oil application is associated with a decrease in TFC below control levels for all the fish species. CSO is associated with the least levels of TFC (3.52 LogCFU/g) in *H. niloticus*. SBO recorded with the least TFC (3.78 LogCFU/g) in *P. annectens* and GNO is associated with the least TFC (3.72 LogCFU/g) in *C. gariepinus*.

In Figure 6, the TFC on all the species increased with increasing duration of storage. *H. niloticus* had the highest TFC (3.89 LogCFU/g) after 8 weeks of storage. *P. annectens* had the highest TFC (4.21 LogCFU/g) after 8 weeks of storage and *C. gariepinus* had the highest TFC of 4.15 LogCFU/g after week 8.

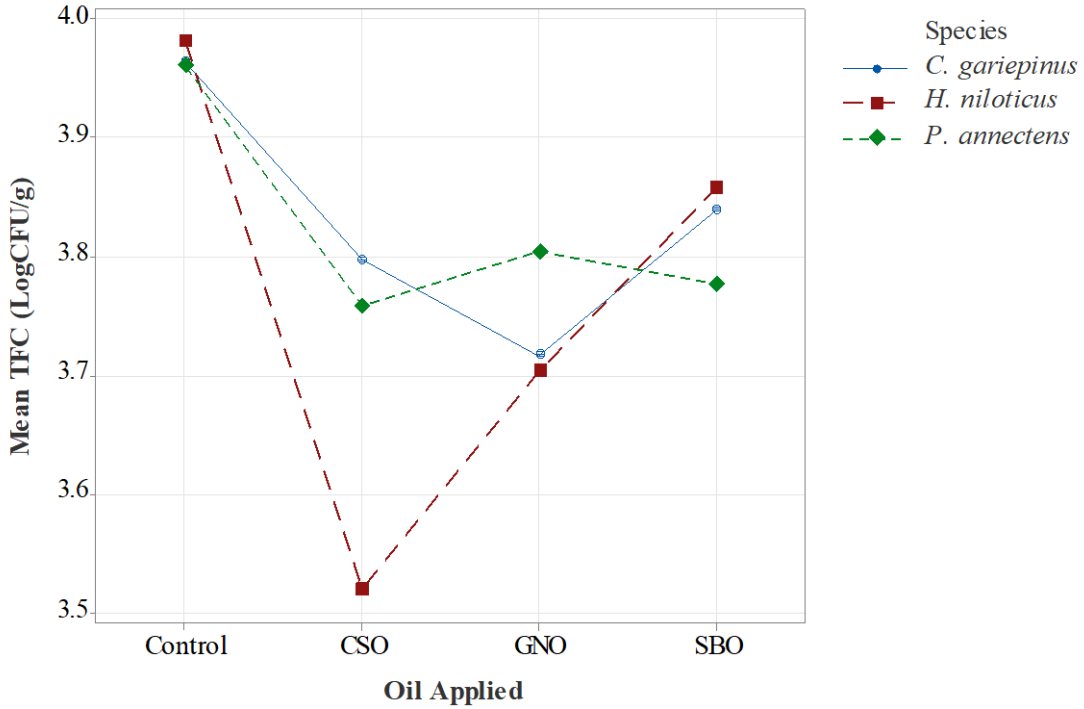

Figure 5: Interaction Plot of Species versus TFC as Determined by oil Treatment

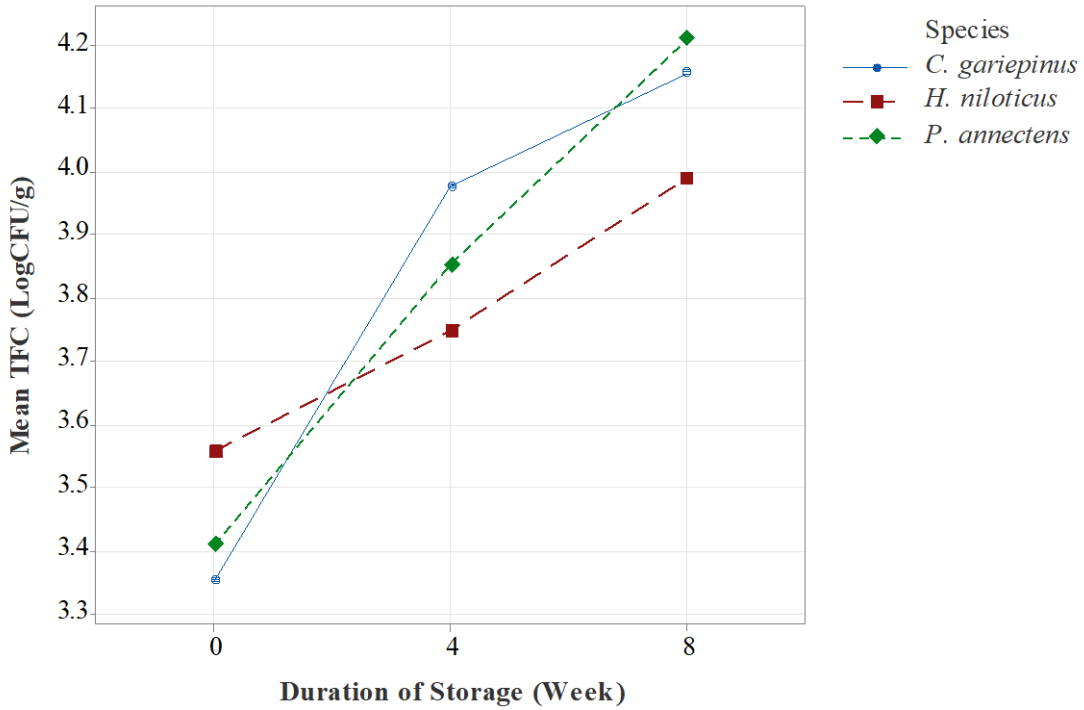

Figure 6: Interaction Plot of Species versus TFC as Determined by the Duration of Storage

DISCUSSION

The Total Viable Count (TVC), a standard measurement of culturable bacteria, is widely used in domestic and international markets to indicate the quality of fresh, frozen and cold-smoked fish (ICMSF, 1986), for example $<10^7$ TVC/g is acceptable for fresh fish (ICMSF, 1986 and Microbiological Guideline for Ready to –eat – Food, 2007). The mean values of TVC in treatment in all samples show similar trend with the observation of Akinwumi and Adegbehingbe (2015) studied microbiological profile of three smoked fish in Ondo State, Nigeria. The authors recorded (4.30 LogCFU/g) for dried catfish and (4.70 LogCFU/g) for dried herring. The increase in mean TVC values in all the samples as storage time increased suggests that the oil treatment had littleor no effect on the proliferation of the microorganisms. However, the lower microbial count observed in castor oil treated samples could be attributed to its higher viscosity compared to the groundnut oil. Adadu *et al.,* (2021) buttressed this in their study of effect castor and groundnut oil on sensory quality of smoked *C. garie*pinus. The TCC value observed in the present study is in agreement with the report of Akinwumi and Adegbehingbe (2015) that observed a range of the total coliform count of dried tilapia sample between (4.08 LogCFU/g) and (5.14 LogCFU/g). Among the oil treatments applied in the present study, groundnut oil treatment was most effective in retaining the initial level of the total fungal count (TFC) of the smoked *C. gariepinus* as it maintained the level at 3logcfu/g for the storage period of 8 weeks. This was followed by castor oil which retained the level of TFC for 4 weeks of storage. Spoilage by microorganisms is a major part of the quality deterioration of smoked fish during storage (Saludeen and Osibona, 2018). Detection of food contamination by bacteria is a major concern in the food sector (Biswal *et al.,* 2020). The observed Total plate counts (TVC) of the smoked fish during storage at ambient temperature given in the present study were found to be increasing as the storage period increased. Patience *et al*., (2019) observed a significant difference in the mycoflora counts of smoked fish from different markets in Nigeria. Similar results were observed by Likongwe *et al*. (2019) with respect to total viable bacterial counts of smoked catfish (6.75 LogCFU/g), (6.28 LogCFU/g), respectively). A higher count of fungal counts isolated in smoked fish upon storage is similar to the observation made on *Oreochromis mossambicus* and *Pangasius hypophthalmus* during storage by Dutta *et al*. (2018). Similar fungal contaminant was observed in smoked catfish and reported by Chukwuemeka *et al*. (2020), The microbial population for the entire treated smoked fish samples observed in this study before storage were within the recommended limits for good quality fish products according to ICMSF (1986) and Microbiological Guideline for Ready to –eat – Food (2007). Observations from the present study are similar to earlier investigation of the effect of natural preservatives on the organoleptic characteristics and storage stability of smoked *Heterotis niloticus* by Amuneke *et, al.* (2020). They recorded total viable count (TVC) of (3.4×10^5 cfu/ml) to (7.8×10^5cfu/ml), and values were regarded to be above the recommended WHO Standard (1.0×10^3 cfu/ml).From the present study, the increase in mean values of TCC in treated smoked *H. niloticus* may be due to the culture in polluted water or due to the unhygienic handling, using of polluted water during processing and improper storage .In this research, the high total coliform count (TCC) observed in treated smoked *H. niloticus* after 8 weeks of storage, which may be due to the use of polluted water during processing, improper post-harvest technology contamination by human or other warm blooded animal's excreta. Majumdar *et al.* (2014) found that the total coliform counts in different species of marine fish samples ranged from (2.18×10^5 LogCFU/g) to (4.18×10^6 LogCFU/g).

The observed microbial loads in the present study agrees with the findings of (Abigaba *et al.,* 2021) on microbiological quality of traditionally smoked fish. The acceptable TVC load in smoked fish does not exceed $<10^7$ CFU/g (ICMSF (1986) and Microbiological Guideline for Ready to –eat – Food, 2007). This study is in agreement as all smoked fish samples examined had TVC load within this value. This indicated good microbiological quality of smoked fish from the processing sites. The low load was probably due to right temperature usage, good personal hygiene, as well as avoided water and equipment contamination. Nevertheless, as indicators of hygiene and contamination (Gilbert *et al*., 2000), the high total coliform load above the permissible limit of 2 CFU/g (ICMSF, 1986) for both untreated (control 3) and treated smoked *P. annectens* samples indicated a likelihood of poor handling during smoking

CONCLUSION

The oil treatments have shown a good potential in controlling microbial population and pathogens in the smoked fish by maintaining microbial counts at safe level during storage. Oil application is associated with a decrease in total microbial count however increasing storage duration is associated with increase total count in all samples evaluated

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