

**Research Article****EFFECT OF FROZEN STORAGE ON MICROBIAL LOAD OF HYBRID *Heteroclaris*, *Clarias gariepinus* AND *Oreochromis niloticus*****A. A. Ayeloja<sup>1\*</sup>, W. A. Jimoh<sup>1</sup>, M. O. Shittu<sup>2</sup>, and B. O. Batatunde<sup>1</sup>**<sup>1</sup>Department of Aquaculture and Fisheries, University of Ilorin, PMB 1515 Ilorin, Kwara State, Nigeria<sup>2</sup>Department of Fisheries Technology, Federal College of Animal Health and Production Technology Moor Plantation, Ibadan, Nigeria\*Corresponding author: ayeloja2@gmail.com, [ayeloja.aa@unlorin.edu.ng](mailto:ayeloja.aa@unlorin.edu.ng)**ABSTRACT**

Effect of frozen storage on microbial load of hybrid *Heteroclaris*, *Clarias gariepinus* and *Oreochromis niloticus* was studied. Fifty samples, each of *Heteroclaris*, *C. gariepinus* and *O. niloticus* with an average weight of  $210 \pm 15$ g were collected at a commercial fish farm in the study area after which they were processed and frozen at  $-18^{\circ}\text{C}$  and microbial analyses were done at 0, 14, 28, 42 and 56 days after frozen storage. Data obtained were logarithmically transformed (log cfu/g) and then subjected to statistical analysis using SPSS 16.0 version. No significant ( $p > 0.05$ ) difference was found for total viable count (TVC), total fungal count (TFC), total coliform count (TCC) and *Klebsiella* spp. count of the fish species studied during the frozen period. The potential of freezing as a good fish preservation method was established as it inhibited microbial activities thereby elongating fish shelf life. It was concluded that *Clarias gariepinus* and *Oreochromis niloticus* fish species can be kept safe up to 56 days by freezing as the microbial loads did not change significantly during the period. Uncontrolled discharge of effluents into the surrounding water bodies should also be checked to avoid contamination prior to fish harvest.

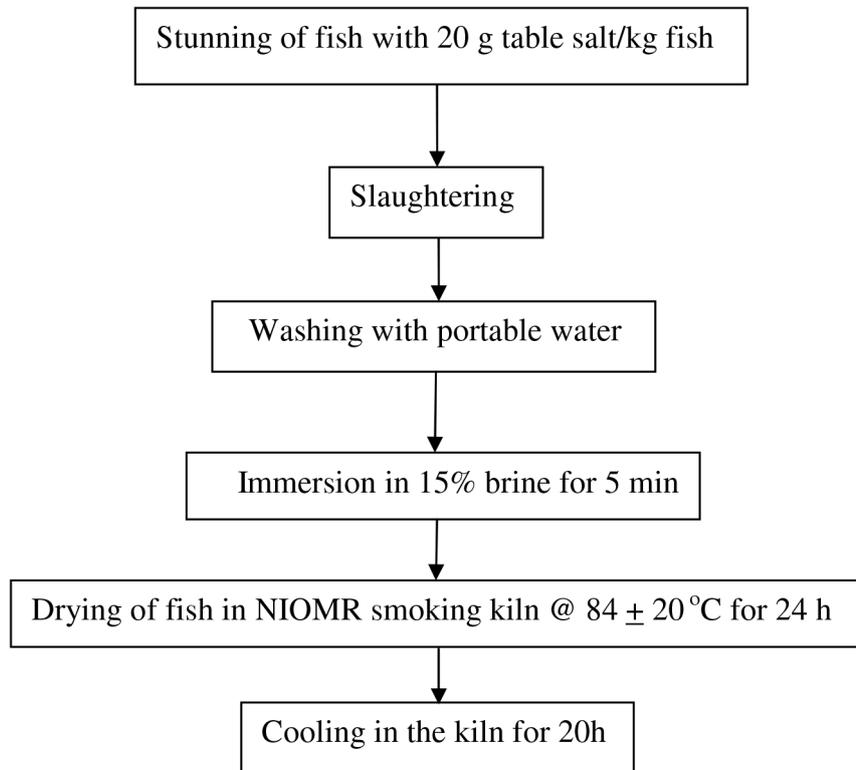
**Key words:** *Clarias gariepinus*, frozen storage, hybrid *Heteroclaris*, microbial load, and *Oreochromis niloticus***INTRODUCTION**

Fish is one of the most important sources of animal proteins and other important elements in the tropics for maintaining good health of people (Al-Jufaili & Opara, 2006). Fish is regarded as healthy food for human because it contains good quality long chain polyunsaturated fatty acids, essential amino acids, vitamins and minerals (Ayeloja, 2019). Warm water fish is generally cheaper and more abundant when compared with fresh water fishes, which are relatively more expensive in Nigeria (Oluwaniyi & Dosumu, 2009). The quality of fish generally decreases after death due to microbiological spoilage and other chemical reactions. Therefore, to reduce postharvest and economic losses, fresh fish should be processed immediately after capture, or left alive for a reasonable period. Several chemical and biological changes take place in the dead fish which can ultimately lead to rejection for human consumption (Agomuo et al., 2017). In Nigeria, fish is eaten fresh, preserved or processed (smoked) and form a much-cherished delicacy that cuts across socio-economic, age, religious and educational barriers (Adebayo-Tayo et al., 2008). Catfishes of the family Claridae comprise the most commonly cultivated fishes in Nigeria (Adewumi & Olaleye, 2011) while Ayeloja et al. (2013) reported that *Sarotherodon galilaeus*, *Oreochromis niloticus* and *Tilapia zilli* which belong to the family Cichlidae as well as *Clarias gariepinus*, *Clarias anguillaris* and *Heterobranchus longifilis* which belong to the family Claridae are among the species of freshwater fish that are mostly utilized in aquaculture, especially in the developing world. *Heteroclaris*, a hybrid of male *Heterobranchus longifilis* and female *Clarias gariepinus*, is more cultured in developing countries now due to its superior growth, improved survival and general hardiness than the pure breed of its parents (Obe, 2014). Also in Africa, *Heteroclaris* is popular among commercial fish farmers as the fishes are tasty, hardy and tolerant to poor water quality conditions (Ekelemu, 2010). The microbial flora associated with freshly harvested fish is principally a function of the environment in which the fish are caught and not of the fish species; hence, the indigenous microbial populations of fish can vary significantly (Shewan, 2000). Contamination of hands and surfaces during cleaning and evisceration of fish is a common route of pathogen infection of fish during processing and storage. Increase in the ambient temperature triggers favourable conditions for microorganisms to thrive, which reduces quality of fish and its potential keeping time leading to food loss (Abolagba & Uwagbai, 2011). Maintaining quality of fish in storage is very necessary for good health of consumers. Under this context a study was done to analyze the effect of frozen storage on microbial load of hybrid *Heteroclaris*, *Clarias gariepinus* and *Oreochromis niloticus* to help establish freezing technique as a potentially good fish preservation method.

**MATERIAL AND METHODS****Sample collection**

Fifty samples each of *Heteroclaris*, *Clarias gariepinus* and *O. niloticus* (average weight  $210 \pm 15$ g) were collected at a commercial fish farm within Ilorin Metropolis, Kwara State, North-Central Nigeria. They were taken

to a laboratory where they were gutted, washed and smoked as modified from the method of Ayeloja et al. (2015) (Figure 1). They were then frozen at a temperature of  $-18^{\circ}\text{C}$ . The microbial analyses were carried out at 0, 14, 28, 42 and 56 days after frozen storage. The experiment was conducted by using a completely randomized design (CRD) with each fish species replicated thrice per storage time by considering storage time the main effect.



**Figure 1. Flow chart for the production of smoked catfish, *Clarias gariepinus* (modified from Ayeloja et al., 2015)**

### Microbial analysis

Total viable count (TVC), total fungal count (TFC), total coliform count (TCC) and *Klebsiella* spp. count were determined using routine microbiological procedures described by Fawole & Osho (1995).

Ten g of each fish sample were weighed aseptically and homogenized in 90ml sterile peptone water. Serial dilutions were made by mixing 1.0ml of the suspension in 9.0ml sterile peptone water to obtain  $10^{-1}$  dilution. The dilution was then made to  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$  and  $10^{-7}$  diluents, then spread-plated onto plates of nutrient agar (for total viable counts); Sabouraud Dextrose agar (SDA) (for total fungal counts), Eosin Methylene Blue agar (for total coliform counts) and MacConkey agar (for *klebsiella* spp.) in triplicates. The plates were incubated at  $37^{\circ}\text{C}$  for 24 hours. Total number of cells per gram of samples was estimated after counting the colonies on the plates.

### Statistical analysis

All data on microbial counts were logarithmically transformed ( $\log \text{cfu/g}$ ) and then subjected to analysis of variance (ANOVA) while means of the significantly different indices were separated using Duncan's multiple range test (DMRT) at  $p < 0.05$ , using SPSS 16.0 version.

## RESULTS

The results in Tables (1, 2 and 3) are not significantly different ( $p > 0.05$ ) for microbial loads (TVC, TFC, TCC and *Klebsiella* spp.) of the fish species through the period of frozen storage at freezing temperature ( $-18^{\circ}\text{C}$ ). The results on Table (1) indicates that the TVC, TFC and TCC for Heteroclaris ranged between 7.111 and 7.371  $\log_{10}$  (CFU/g), 7.588 and 6.820  $\log_{10}$  (CFU/g), and 7.117 and 7.584  $\log_{10}$  (CFU/g), respectively. Similar trend was observed for the microbial load of *C. gariepinus* and *O. niloticus*.

**Table 1. Mean value of total viable count (TVC), total fungal count (TFC), total coliform count (TCC) and *Klebsiella* spp. count from frozen *Heteroclaris***

| Storage time | TVC<br>$\log_{10}$ (CFU/g) | TFC<br>$\log_{10}$ (CFU/g) | TCC<br>$\log_{10}$ (CFU/g) | <i>Klebsiella</i> spp. $\log_{10}$<br>(CFU/g) |
|--------------|----------------------------|----------------------------|----------------------------|---|
| Day 0        | 7.111 ± 0.59               | 6.766 ± 0.66               | 7.117 ± 0.66               | 7.121 ± 0.56                                  |
| Day 14       | 7.277 ± 0.54               | 6.820 ± 0.65               | 7.323 ± 0.60               | 7.331 ± 0.61                                  |
| Day 28       | 7.340 ± 0.59               | 6.588 ± 0.58               | 7.449 ± 0.68               | 7.323 ± 0.56                                  |
| Day 42       | 7.537 ± 0.62               | 6.787 ± 0.55               | 7.490 ± 0.66               | 7.475 ± 0.61                                  |
| Day 56       | 7.371 ± 0.60               | 6.809 ± 0.63               | 7.584 ± 0.67               | 7.542 ± 0.63                                  |

Note: Mean values are not significantly different ( $p > 0.05$ ) in all the columns.

**Table 2. Mean value of total viable count (TVC), total fungal count (TFC), total coliform count (TCC) and *Klebsiella* spp. count from frozen *Clarias gariepinus***

| Storage time | TVC<br>$\log_{10}$ (CFU/g) | TFC<br>$\log_{10}$ (CFU/g) | TCC<br>$\log_{10}$ (CFU/g) | <i>Klebsiella</i> spp.<br>$\log_{10}$ (CFU/g) |
|--------------|----------------------------|----------------------------|----------------------------|---|
| Day 0        | 7.588 ± 0.55               | 6.851 ± 0.57               | 7.595 ± 0.57               | 7.4111 ± 0.61                                 |
| Day 14       | 7.438 ± 0.62               | 6.938 ± 0.65               | 7.316 ± 0.60               | 7.288 ± 0.62                                  |
| Day 28       | 7.700 ± 0.62               | 6.863 ± 0.59               | 7.223 ± 0.55               | 7.569 ± 0.60                                  |
| Day 42       | 7.608 ± 0.55               | 6.639 ± 0.51               | 7.381 ± 0.64               | 7.438 ± 0.62                                  |
| Day 56       | 7.615 ± 0.56               | 6.688 ± 0.65               | 7.433 ± 0.65               | 7.427 ± 0.44                                  |

Note: Mean values are not significantly different ( $p > 0.05$ ) in all the columns.

**Table 3. Mean value of total viable count (TVC), total fungal count (TFC), total coliform count (TCC) and *Klebsiella* spp. count from frozen *Oreochromis niloticus***

| Storage time | TVC<br>$\log_{10}$ (CFU/g) | TFC<br>$\log_{10}$ (CFU/g) | TCC<br>$\log_{10}$ (CFU/g) | <i>Klebsiella</i> spp.<br>$\log_{10}$ (CFU/g) |
|--------------|----------------------------|----------------------------|----------------------------|---|
| Day 0        | 7.379 ± 0.61               | 6.594 ± 0.63               | 7.321 ± 0.65               | 7.275 ± 0.60                                  |
| Day 14       | 7.426 ± 0.67               | 6.613 ± 0.66               | 7.358 ± 0.64               | 7.338 ± 0.62                                  |
| Day 28       | 7.458 ± 0.66               | 6.659 ± 0.64               | 7.387 ± 0.59               | 7.376 ± 0.65                                  |
| Day 42       | 7.500 ± 0.66               | 6.739 ± 0.62               | 7.463 ± 0.61               | 7.496 ± 62                                    |
| Day 56       | 7.533 ± 0.65               | 6.838 ± 0.62               | 7.501 ± 0.61               | 7.539 ± 0.62                                  |

Note: Mean values are not significantly different ( $p > 0.05$ ) in all the columns.

## DISCUSSION

The results of this study clearly showed that freezing inactivated microbial activities and thereby elongating the shelf life of the fish during frozen storage. This is in line with the findings of Samah and Samia (2014) in which super chilling did cause no significant increase in the microbial load of Tilapia and Nile perch. Low temperature slows down all biochemical, physical, and microbiological process of food products (Ayeloja et al., 2018). As per the standard of the International Commission on Microbiological Specification for Food (ICMSF, 1986), the minimum recommended acceptable limit is 5.0  $\log_{10}$  CFU/g for good quality product (Ayeloja et al., 2011). However, the microbial loads obtained in this study ranged from 6.5 to 7.7  $\log_{10}$  CFU/g. This could be attributed to contamination from source prior to storage. Large domestic effluents are discharged to Egbeji river in Ilorin and the river is the main source of water used to feed the ponds in raising fish in the study area. Daramola et al. (2014) reported high level of the microbial content in *C. gariepinus* due to polluted ponds. Similar situations exist in other areas (Ayeloja & Adeoye, 2018; Shamsuzzaman et al., 2011). Also Ayeloja et al. (2011) reported that fish farmers are not usually careful when handling fish after harvesting as freshly caught fish are usually covered with damp sacks and sometimes they are mixed with wet grass or water weeds to reduce the temperature; any fish treated this way is prone to contamination with microorganisms. Therefore, hygienic handling of fish after harvesting is required so as to avoid contamination of fish right from farm.

## CONCLUSION

Freezing is a good method of fish preservation as it inhibits microbial activities thereby slowing down the process of spoilage. However, it depends on microbial load of fish prior to frozen storage. Polluted ponds and unhygienic handling after harvest contaminate the fish.

## REFERENCES

- Abolagba, O. J., & Uwagbai, E. C. (2011). A Comparative analysis of the microbial load of smoke-dried fishes (*Ethmalosa fimbriata* and *Pseudolithus elongatus*) sold in Oba and Koko markets in Edo and Delta States, Nigeria at different seasons. *Australian Journal of Basic Applied Sciences*, 5(5), 544-550.
- Adebayo-Tayo B. C., Odu, N. N., Anyamele, L. M., Igwiloh, N. J., & Okonko, I. O. (2008). Microflora of smoked-dried fishes sold in Uyo, Eastern Nigeria. *World Journal of Agricultural Science*, 43, 346-350.
- Adewumi, A. A. & Olaleye V. F. (2011). Catfish culture in Nigeria: Progress, prospects and problems. *African Journal of Agricultural Research*, 6 (6), 1281-1285.
- Agomuo, J. K., Ehirin, F., & Onugha, F.C. (2017). Storage stability of smoked mackerel fish (*Scomboromorus scombus*) as affected by selected packaging materials. *World Journal of Food Science and Technology*, 1(1), 22-27.
- Al-Jufaili, M. S., & Opara L. U. (2006). Status of fisheries post-harvest industry in the Sultanate of Oman: Part 1 - Handling and marketing system of fresh fish. *Journal of Fisheries International*, 1(2-4), 144-149.
- Ayeloja, A. A. (2019). Sensory quality of smoked *Clarias gariepinus* (Burchell, 1822) as affected by spices packaging methods. *International Journal of Food Properties*, 22 (1), 704-13. <https://doi.org/10.1080/10942912.2019.1597880>
- Ayeloja, A. A., & Adeoye, Y. D. (2018). Heavy metals concentration in selected fish species from River Dandaru, Ibadan, Oyo State, Nigeria. *Production Agriculture and Technology (PAT) Journal*, 14(2), 87-91.
- Ayeloja, A. A., George, F. O. A., Jimoh, W. A., Shittu, M. O., & Abdulsalami, S. A. (2018). Microbial load on smoked fish commonly traded in Ibadan, Oyo State, Nigeria. *Journal of Applied Science and Environmental Management*, 22, 493-497.
- Ayeloja, A. A., George, F. O. A., Akinyemi, A. A., & Atanda, O. O. (2015). Proximate and mineral composition of spiced smoked catfish *Clarias gariepinus* (Burchell, 1822). *Journal of Agricultural Science and Environment*, 15(2), 68-74.
- Ayeloja, A. A., George, F. O. A., Dauda, T. O., Jimoh, W. A. & Popoola, M. A. (2013). Nutritional comparison of captured *Clarias gariepinus* and *Oreochromis niloticus*. *International Research Journal of Natural Sciences*, 1 (1), 9-13.
- Ayeloja, A. A., George, F. O. A., Obasa, S. O. & Sanni, L. O. (2011). Effect of post-slaughter time intervals on the quality of the African catfish, *Clarias gariepinus* (Burchell, 1822). *American Journal of Food Technology*, 6 (9), 790 - 797.
- Daramola, J. A., Alao, F. O., Osofero, S. A., & Wemimo, O. O. (2014). Assessment of the microbial and sensory qualities of smoked African catfish (*Clarias gariepinus*). *Journal of Agriculture and Environmental Sciences*, 3(1), 11-22.
- Ekelemu, J. K. (2010). Differential growth patterns of *Clarias gariepinus*, *Heterobranchus bidorsalis* and Hybrid Heteroclaris fed commercially prepared diets. *Agriculture and Biology Journal of North America*, 1(4), 658-661.
- Fawole, M. O. & Osho, B. A. (1995). Laboratory manual of microbiology. Shalom prints Ibadan Nigeria. Pp 25-30.
- Obe, B. W. (2014). Growth performance and nutrient utilization of catfish hybrid (*Heterobranchus bidorsalis* X *Clarias gariepinus*) fed fermented sorghum (*Sorghum bicolor*) waste meal diets. *International Journal of Applied Science and Technology*, 4(3), 130-136.
- Oluwaniyi O. O., & Dosumu O. O. (2009). Preliminary studies on the effect of processing methods on the quality of three commonly consumed marine fishes in Nigeria. *Biokemistri*, 21(1), 1-7.
- Samah, H., & Samia, H. (2014). Effect of super chilling on microbial load and chemical composition of Nile tilapia and Nile perch. *Direct Research Journal of Agriculture and Food Science (DRJAFS)*, 2(5), 40-43.
- Shamsuzzaman, M. M., Mazumder, S. K., Siddique, M. A., & Miah, M. N. U. (2011). Microbial quality of hilsa shad (*Tenuulosa ilisha*) at different stages of processing. *Journal of the Bangladesh Agricultural University*, 9(2), 339-344.
- Shewan, J. M. (2000). The microbiology of sea-water fish. In: Fish as Food (Borgstrom, G., ed.). Academic Press, FL, 487-560.