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Full Length Research Paper

Microbiological assessment of stored *Tilapia guineensis*

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Microbiological analysis of *Tilapia guineensis* was assessed at two ice stored temperatures, -18 and 4°C, for 4 weeks. Bacteria and fungi counts decreased from 7.9×10^3 to 5.4×10^1 cfu/g for bacteria and 6.2×10^3 to 3.2×10^2 cfu/g for fungi in the samples stored at -18°C, while for the samples stored at 4°C, an increase was shown in counts from 7.9×10^3 to 7.6×10^7 cfu/g for bacteria and 6.2×10^3 to 6.8×10^4 cfu/g for fungi. *Pseudomonas*, *Staphylococcus*, *Bacillus*, *Proteus*, *Micrococcus* and *Aeromonas* sp. were the bacteria species isolated before cold storage, while only *Pseudomonas*, *Staphylococcus*, *Bacillus* and *Proteus* sp. were isolated from samples stored at -18°C. The same organisms isolated before cold storage were also isolated for samples stored at 4°C. Fungal species isolated before cold storage include: *Cladiosporium, Aspergillus* and *Fusarium* sp. All these fungi species were also isolated from samples stored at -18°C. *Cladiosporium, Pichia, Aspergillus* and *Fusarium* sp. were isolated from samples stored at 4°C. The level of bacteria and fungal growth in fish stored at 4°C temperature exceeded the acceptable microbiological limits (10^2/g for moulds and 10^3/g for bacteria). However, the pH of fish was found to increase in the two stored temperatures. It was within the alkaline range in the -18°C stored samples, but within the acidic range in the 4°C stored samples. There were significant differences ($P<0.05$) in the microbiological composition of the ice stored *Tilapia guineensis* within the same temperature and between the two temperatures. The quality of the 4°C stored sample deteriorated faster than that of the -18°C. Thus, storage temperature and duration have effects on the quality of stored fish.

Key words: *Tilapia guineensis*, fish, sample, bacteria.

INTRODUCTION

Tilapia guineensis is a brackish water euryhaline species found along the West Coast of Africa (Philippart and Ruwet, 1982). There is an increasing interest in this fish for aquaculture purposes, particularly in areas of high or variable salinities, characteristic of the estuaries and extensive lagoon systems which constitute its natural habitat (Akinwumi, 2001; Akinrotimi, 2006). It contains high levels of proteins, water, fat or lipid, and other nitrogenous compounds, as well as mineral component, which make it ideal for human consumption. Like all living things, fish contain microorganisms which degrade them soon after the death of the fish. Fish get spoilt very quickly and man had to develop methods to preserve fish very early in history. The consumption of fresh fish was, in the past, limited to the point of capture because of their highly perishable nature (Frazier and Westhoff, 1991).

Current preservative techniques, such as use of preservatives, chilling, freezing, salting, smoking, canning, drying, or a combination of these methods (Jay, 1996, Chae et al., 2009; Liu et al., 2009), have reduced the problem of perishability of fish and its products. Microbial activity is responsible for spoilage of most fresh

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fish products. The shelf life of fish products, therefore, is markedly extended when products are stored at low temperatures. In industrialized countries, it is common practice to store fresh fish in ice at 0°C (Liu et al., 2009). Olley and Ratkowsky (1973) found that enzymatic and microbial activities are greatly influenced by temperature. However, in the temperature range of 0 to 25°C, microbiological activity is relatively more important, and temperature changes have greater impact on microbiological growth than on enzymatic activity. This is because many bacteria are unable to grow at temperatures below 10°C and even psychrotrophic organisms grow very slowly, and sometimes with extended lag phases, when temperature approaches 0°C. Man prefers to consume fresh fish rather than other types of fish products. The freshness of fish deteriorates with time, until the product is no longer acceptable to the consumer. Quality deterioration of fresh fish is caused by lipid oxidation and microbial spoilage.

Fish has been one of the main foods for humans for many centuries and still constitutes an important part of the diet in many countries. The advantages of fish as a food are its easy digestibility and high nutritional protein value. The present paper reports the microbiological composition and shelf-life of *T. guineensis* stored at two temperatures of -18 and 4°C.

METHODS

Fish samples

Two hundred and sixty *T. guineensis* fish were used for this study; they were divided into two equal groups for -18 and 4°C storage. Each experimental procedure was done with three replicates to eliminate errors. The fish were caught with fishing nets, from the brackish water ponds of the African Regional Aquaculture Centre (ARAC), Buguma, Rivers State, Nigeria. The fish samples were immediately transported in a cooler containing ice blocks to the University of Port Harcourt where they were stored at a temperatures of 4 and -18°C for 4 weeks.

Preparation of the sample for microbiological analysis

Ten grams of flesh and gills of the iced fish were collected using a dissecting set. These were separately added to 90 ml of 0.1% peptone water and homogenized in a blender. 1 ml of the homogenate was transferred to a test tube containing 9 ml peptone water to obtain a dilution of 10^{-1}. In a similar manner, 1 ml was transferred from this dilution to a test tube containing 9 ml diluents and the process was repeated until a dilution of 10^{-9} was obtained according to the method of Esther et al. (2010).

Enumeration of micro organisms

From each dilution, 0.1 ml was transferred to plates of nutrient agar (Titan Biotech, New Delhi, TM 1038) for bacteria and malt extract agar (Titan Biotech, New Delhi, TM 382) and for fungi in triplicates using the spread plate technique. Plates, containing nutrient and malt extract agar, were incubated at 37°C for 18 to 24 h and 28°C for 2 to 3 days, respectively. Counts were made after incubation from plates having 30 to 300 colonies according to the method of Ashok (2008).

Identification of bacterial isolates

All colonies having characteristic edges, colors and sizes were viewed with a microscope, then isolated and purified on malt extract agar for fungi, and nutrient agar for bacteria. Each isolate was subjected to a biochemical test using the Bergey's manual of systematic bacteriology according to Bergy and Holt (1994) and the fungal isolates were identified according to the methods of Treagen and Pulliam (1982).

Total viable count

The plate count agar inoculated with bacterial isolates was incubated at 37°C for 48 h. After 48 h, the colour, size, shape, texture surface elevation and margin of the colonies were observed with a microscope and recorded. The numbers of the colonies were counted, and the colony forming units per gram (cfu/g) were calculated and recorded according to the methods of Olafsdottir et al. (1997).

pH determination

The pH of fish flesh was measured using a pH meter (PHS-3C, Shangai, China) after blending 10 g of homogenized fish with 100 ml of distilled water.

Statistical analysis

The experiment was conducted in triplicates. The results were reported as mean values ± standard deviation. The student's t-test was used to find out the significance between different treatments and storage periods as described by Telma et al. (2008). The differences between the mean values were considered significant when $P<0.05$. All data were analysed using SAS statistical package for windows (SAS, 2009).

RESULTS

Bacterial counts

Bacterial counts on *T. guineensis* stored at -18°C decreased during the four weeks storage period. The decrease ranged from 7.9×10^3 cfu/g at the time of storage to 5.4×10^1 cfu/g at the end of four weeks, while bacterial counts on *T. guineensis* stored at 4°C increased from 7.9×10^3 at storage time to 7.6×10^7 after four weeks (Table 1). Biochemical characterization of bacteria isolates revealed that *Pseudomonas*, *Staphylococcus*, *Bacillus, Proteus, Micrococcus* and *Aeromonas* sp. were isolated from *T. guineensis* before storage. At the end of four weeks, *Pseudomonas*, *Staphylococcus*, *Bacillus* and *Proteus* sp. were isolated from *T. guineensis* after storage at -18°C, while *Micrococcus* and *Aeromonas* sp. were not isolated. For the 4°C stored samples, *Pseudomonas*, *Staphylococcus*, *Bacillus, Proteus, Micrococcus* and *Aeromonas* sp. were isolated from

Table 1. Bacterial counts (cfu/g) of *T. guineensis* stored at two temperatures.

Storage time (weeks)	18°C	4°C
0	7.9×10^{3aA}	7.9×10^{3dA}
1	7.3×10^{2aA}	8.2×10^{4cB}
2	2.1×10^{2bA}	2.3×10^{6bB}
3	8.9×10^{1cA}	4.8×10^{6bB}
4	5.4×10^{1bcA}	7.6×10^{7aB}

[a-d] Different letters in the same column indicate significant difference (P<0.05). [A-B] Different letters in the same row indicate significant difference (P<0.05).

Table 2. Occurrence of bacterial isolates from *T. guineensis* after 4 weeks of cold storage at -18 and 4°C.

Sample code (c)	Bacterial isolate	Occurrence		
		Before storage	After 4 weeks storage	
			-18°C	4°C
TG1	*Pseudomonas* sp.	+	+	+
TG2	*Staphylococcus* sp.	+	+	+
TG3	*Proteus* sp.	+	+	+
TG4	*Bacillus* sp.	+	+	+
TG5	*Micrococcus* sp.	+	-	+
TG6	*Aeromonas* sp.	+	-	+

+ = present, - = absent.

Table 3. Fungal counts (cfu/g) of *T. guineensis* stored at two temperatures.

Storage time (weeks)	-18°C	4°C
0	6.2×10^{3aA}	6.2×10^{3bA}
1	4.2×10^{2bA}	4.0×10^{2cA}
2	3.1×10^{2cA}	4.9×10^{3bcB}
3	3.7×10^{2bA}	5.3×10^{3bB}
4	3.2×10^{2cA}	6.8×10^{4aB}

[a-c] Different letters in the same column indicate significant difference (P<0.05). [A-B] Different letters in the same row indicate significant difference (P<0.05).

T. guineensis after storage for 4 weeks. The bacteria species remained the same as those isolated before cold storage (Table 2). Statistical analysis revealed significant differences (P<0.05) in bacterial counts within the same storage temperatures and between the two temperatures during the storage period.

Fungal counts

Fungal counts on *T. guineensis* stored at -18°C decreased during the four weeks storage period. The decrease ranged from 6.2×10^3 cfu/g at the time of storage to 3.2×10^2 cfu/g at the expiration of four weeks. On the contrary, fungal counts on *T. guineensis* stored at 4°C increased to 6.8×10^4 at the end of four weeks (Table 3).

Cladiosporium, Aspergillus and *Fusarium* sp. were isolated from *T. guineensis* before cold storage (Table 4). At the end of four weeks, *Cladiosporium, Aspergillus* and *Fusarium* sp. were isolated from *T. guineensis* after storage at -18°C (Table 4). *Cladiosporium, Pichia, Aspergillus* and *Fusarium* sp. were isolated from *T. guineensis* after storage at 4°C for 4 weeks (Table 4). Statistical analysis revealed significant differences (P<0.05) in bacterial counts within the same storage temperatures and between the two temperatures.

pH

There was a decrease in the pH levels of the fish species with time. The -18°C sample decreased in pH from 6.81

Table 4. Occurrence of fungal isolates from *T. guineensis* after 4 weeks cold storage at -18 and 4°C.

Sample code (c)	Fungal isolate	Occurrence		
		Before storage	After 4 weeks of storage	
			-18°C	4°C weeks
TG7	*Cladiosporium* sp	+	+	+
TG8	*Aspergillus* sp	+	+	+
TG9	*Fusarium* sp	+	+	+
TG10	*Pichia* sp	-	-	+

+ = present, - = absent.

Table 5. pH levels of ice stored *T. guineensis*.

Storage time (Weeks)	Storage temperature	
	-18°C	4°C
0	6.81±0.41^{aA}	6.81±0.14 ^{aA}
1	6.75±0.27^{aA}	6.90±0.12 ^{aA}
2	6.75±0.32^{aA}	7.10±021^{bB}
3	6.80 ±0.16^{aA}	7.15±0.24^{bB}
4	6.85±0.33 ^{aA}	7.20±0.33 ^{bA}

$^{a-b}$ Different letters in the same column indicate significant difference (P<0.05). $^{A-B}$ Different letters in the same row indicate significant difference (P<0.05).

before cold storage to 6.75 at the expiration of one week, but subsequently, it increased to 6.85 at the end of four weeks. The 4°C sample increased in pH from 6.81 before cold storage to 7.20 at the expiration of four weeks. The fish sample was observed to be more acidic with increase in storage time. Statistical analysis revealed significant differences (P<0.05) in pH levels of the fish species stored at the 4°C temperature and between the two temperatures (Table 5).

DISCUSSION

Generally, the trend in the world today is towards healthy eating more than ever. People are more conscious of what they eat, associating same with health conditions presently or in later years of life. One area where this is observed is in the change from the consumption of red meat due to its high cholesterol content to white meat and fish. *T. guineensis* was chosen for this study because of its high demand which was attributable to its good taste and nutritional composition that met the nutritional requirements of many consumers. This study revealed that freezing resulted in lower microbial counts for the fish samples stored at -18°C as compared to fish samples that were chilled at 4°C.

During storage, the aerobic plate count (APC) values of the -18°C stored samples were always lower than the 4°C stored samples. The increase in bacteria and fungal counts in the 4°C indicate that the microorganisms were able to proliferate at this temperature, while the -18°C frozen fish samples showed a consistent reduction in the aerobic plate counts (APC) of the bacteria and fungi. The APC in the 4°C stored samples were observed to increase with increase in storage time. Aerobic plate counts indicate the level of microorganisms in a product, but for fish and fishery products, they generally do not relate to food safety hazards, but sometimes can be useful to indicate quality shelf life and post heat processing contamination (Maturin and Peeler, 1998).

The changes in bacteria and fungi population during the study is in agreement with works of (FDA, 1998) which reported that the storage temperature of fresh fish samples and the plating medium used can affect the number and type of bacteria isolated. This is due to the variation in temperature requirement for proliferation and the nutrient and salt requirement for the various micro-organisms. This is also in agreement with Rong et al. (2009) who reported changes in microbial isolates of ice stored Pacific oysters stored at different temperatures. The changes were associated with the fact that *Shewanella* spp constituted a low initial proportion resulting in the ability of *Pseudomonas* sp. to inhibit the H_2S producing bacteria. Koutsoumanis et al. (1999) report that *Pseudomonas* and *Shewanella* spp are two strongly competitive psychrotrophic microorganisms, while Gram and Melchiorsen (1996) report that *Pseudomonas* spp has strong inhibitory effect on other organisms due to its ability to produce siderophores.

The variations in the isolated bacteria and fungal

species before and after cold storage for four weeks may be due to the changes in chemical, proximate composition and pH of the fresh fish associated with storage temperatures, to which different bacteria and moulds react differently (Fafioye et al., 2002).

Pseudomonas, *Staphylococcus*, *Proteus*, *Bacillus*, *Micrococcus* and *Aeromonas* spp were the bacteria species isolated before cold storage at the two temperatures. These same organisms were isolated at the expiration of four weeks from the 4°C stored fish samples, while only *Pseudomonas*, *Staphylococcus*, *Proteus* and *Bacillus* spp, were isolated from the -18°C stored fish samples. *Cladiosporium*, *Aspergillus* and *Fusarium* spp were the isolated fungal species before cold storage. At -18°C stored temperature, the same organisms were isolated from the fish samples, while at the 4°C stored temperature, *Cladiosporium*, *Aspergillus*, *Fusarium* and *Pichia* spp were isolated. This is associated with the biochemical changes occurring in the flesh of the fish making it vulnerable to the growth of the *Pichia* spp yeast, whose presence may also be associated with fermentation causing changes in the biochemical composition of the flesh of the ice stored fish, which may have subsequently resulted in the increase observed in the pH.

The pH of fish stored at the -18°C temperature was observed to initially drop from 6.81 before ice storage to 6.75 after one week storage, after which there was a subsequent increase to 6.85 after four weeks. The pH increased with increasing storage time, and showed same pattern at the 4°C storage. The increase was higher in the 4°C stored samples than in the -18°C stored sample, indicating that biochemical and microbial changes were occurring faster in the flesh of 4°C stored fish even at these temperatures. The increase observed in pH in this study can also be attributed to the fact that fermentation of carbohydrate to acid was occurring. Eyo (2001) showed that pH was an indication of the extent of microbial spoilage in fish. He stated that some proteolytic microbes produce acids after decomposition of carbohydrates, thereby increasing the acid level of the fish. The increase in pH may also indicate the accumulation of alkaline compounds, such as ammonia, mainly derived from microbial actions. The increase may also be due to an increase in volatile bases from the decomposition of nitrogenous compounds by endogenous or microbial enzymes (Erkan and Ozden, 2008).

pH, as an index, is important in determining the quality of fish, and can be used as a guide (Pacheco-Aguilar et al., 2000). The pH of fish flesh and gills has an important influence on its freshness because of its influence on bacterial growth as found in this study. Consequently, the lower the pH of the fish flesh and gills, the slower the bacteria growth, and vice versa (Okeoyo et al., 2009).

Based on microbiological recommendations of the food and agricultural organization (FAO, 1992), fish samples stored at -18°C remained within the acceptable microbiological limits for bacteria and fungi, while fish stored at the 4°C exceeded the recommended microbiological limits for bacteria and fungi. Thus, in this case, the consumption of fish stored at the 4°C temperature may be hazardous to the consumer.

Conclusion

Freezing of fish at -18°C reduces spoilage than freezing at 4°C temperature. This study reveals that even at these temperatures, as the storage period increases, there are relative changes in the microbial composition that has direct effect on the shelf life and market value of the fish. Freezing of fish at -18°C creates an unfavourable environ-mental condition for the growth and survival of the microorganisms found on this fish, thereby increasing the shelf life, while freezing at 4°C temperature allows for the proliferation of the microorganisms, thereby reducing the shelf life. For cold storage temperatures, -18°C is recommended, since it will increase the shelf life of the fish than 4°C temperature. Though deterioration of the fish sample was obvious from the microbiological analysis, it was faster in the 4°C stored fish samples than in the -18°C stored fish samples. This study has shown that the shelf life of *T. guineensis* can be remarkably extended if properly stored. Thus, it is advisable that fresh fish for consumption stored under freezing conditions of -18°C should be used within one week of capture to harness the fresh quality state of the fish and avoid changes that may be detrimental to the consumer.

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