Quality Assessment of a common Nigerian Marine Fish, Croaker (*Pseudotolithus elongatus*) under different Storage Conditions

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Abstract: Quality assessment of a common Nigerian marine fish, Croaker (*Pseudotholitus elongatus)* was carried out at various storage temperatures such as ambient (28^{o}C), Refrigeration (4 ^{o}C) and Frozen state (-5 ^{o}C) using sensory, microbiological and biochemical method of evaluation. At ambient temperature, the shelf life of the fish was estimated to be 12hrs. At this time, the fish was still sensorily acceptable, the bacterial load of the tissue was 2.7 x 10^4 cfu/g, TMA-N and TVB-N values were 5.2 mg/100g and 24.8mg/100g respectively while the pH was 7.1. At refrigeration temperature (4^0C), the shelf life of the fish sample was estimated to be 6 days. At this time, the fish was still sensorily acceptable, the bacterial load of the tissue was 7.1 x 10^4 cfu/g, TMA-N and TVB-N values were 5.60mg/100g and 32.90mg/100g respectively while the pH was 6.8. At freezing temperature (-5^0C), the fish was still sensorily acceptable at the estimated shelf life of 3 weeks. The bacterial load of the tissue was 6.3 x 10^3 cfu/g. TMA-N and TVB-N values were 7.50mg/100g and 30.80mg/100g respectively while the pH was 7.1. The limits of freshness stated here were mostly within the proposed limits of acceptability by some international quality standard organisations for marine fishes. [New York Science Journal 2010;3(8):29-36]. (ISSN: 1554-0200).

Keywords: Marine fishes, *Pseudotolithus elongatus*, quality assessment, shelf life and storage temperature.

1. Introduction:

Fresh Fish are among the most perishable food products known to man and the monitoring and control of fish quality is one of the main goals in the fish industry. Fish shelf life is influenced by a number of factors such as initial microbiological load, season, handling and feeding and the limited and variable shelf lives of Fish are major problems for Fish quality and assurance (Konstantinos, 2001).

According to Huss(1995), the main changes that can occur between capture and consumption of fish can be divided into 3 stages;

i. The pre-rigor state in which the muscle tissue is soft and pliable.
ii. The stiff and rigor condition known as rigor mortis whose onset can occur between 1-24hrs depending on the fish specie following the death of fish.
iii. The post-rigor state in which the fish softens and starts to deteriorate.

It is noteworthy that pollution can also significantly affect the shelf life of the marine fish because the fish species inhabiting polluted waters have already compromised their original healthy status (Adeyemo, 2003).

Generally, In the first couple of hours after death, changes in fish are mainly due to biochemical processes, however several hours after death, few bacteria can penetrate the flesh where they degrade tissue components producing the unpleasant odours and flavours that are associated with spoilage (Huss, 1995). Fish spoilage is as a result of autolysis, oxidation and bacterial growth or by a combination of these. Bacterial spoilage of Fish does not begin until after rigor mortis when the juices are released from the Fish fibres, therefore the more rigor is delayed, the longer the keeping quality of Fish (Frazier and Westhoff, 1978). Rigor mortis is hastened by struggling of the fish, lack of oxygen and warm temperature and is delayed by low pH and adequate cooling of the Fish (Frazier and Westhoff, 1978). Time and temperature are therefore the most critical factors to control to ensure that seafood retains high freshness quality as long as possible (Adams and Moses, 2008). At room temperature, seafood are likely to deteriorate very fast, Freezing and storage at low temperatures slows down bacterial growth and deterioration of fish through some enzymatic and chemical changes that progresses slowly (Huss, 1988).

Quality assessment of fishes has more to do with the determination of its shelf life or storage life which is the amount of time that sea foods remain palatable. Different species of fishes have different shelf lives which also varies depending on the oil levels, catch area, season, duration of rigor mortis, intrinsic conditions of the fish and how it was captured and handled (Huss, 1995). The shelf life of most marine

fishes have been predicted to range between 2-24 days in ice, 5 days at 5°C and 3 days at 10 °C (Huss, 1995). The shelf life of Croaker (*Pseudotolitus elongatus*) found in Nigerian marine waters have been predicted to be 20 days in ice and 12 hrs at ambient temperature (Ola and Oladipo, 2004). Super chilling at -4°C and below have proven to extensively extend the shelf life of frozen fish to several weeks because at such temperatures, microbial spoilage is almost impossible, its only chemical and enzymatic changes that can lead to spoilage after a considerable long time (Huss, 1995. Adams and Moses, 2008).

Marketing of Fish in Nigeria is mostly carried out by local fish sellers at ambient temperature, therefore knowledge of spoilage patterns of tropical fishes and their shelf life under ambient conditions is very important. Refrigeration temperatures are also relevant because they are used by most households in Nigeria for temporary storage of fish. Frozen state condition is also important since most fishes consumed in Nigeria are imported and usually come in frozen state.

In the recent time, modern biotechnology have introduced new techniques that can detect early fish contamination, improve the taste, modify the quality of fish and prolong the shelf life and also impact disease resistance to the fish (William and Michael, 2009).

There are two main methods of assessing fish quality to determine its freshness and shelf life and these are the Sensory and Non-sensory methods. Sensory methods rely mostly on appearance, odour, texture and taste of the Fish while non-sensory methods use physical, biochemical, chemical and microbiological means (Huss, 1995).

In the present study, quality assessment was carried out on a common Nigerian marine fish, Croaker (*Pseudotolithus elongatus)* at different storage temperatures such as ambient (28°C), Refrigeration (4°C) and frozen state (-5 °C) using sensory, Microbiological and Biochemical method of evaluation. The limits of freshness proposed by some international standard organisations for marine fishes were used as standard limits in this study.

2. Materials and Methods:

i. Sample collection:

Fish samples Croaker (*Pseudotholithus elongatus)*, were collected live from the Nigerian Institute for Oceanography and Marine Research (NIOMR) fishing boat, "R.V. Federal Argonaut" on arrival from sea trip. The live fishes were killed by banging then on the head with a hammer and the exact time of death of life fishes were noted. The fishes were then taken to the laboratory for proper identification and analysis.

ii. Storage temperature:

The storage temperature employed were -5°C, 4°C and 28°C. A refrigerator with a temperature control setting was used and it was adjusted to give a temperature of -5°C at the upper freezing chamber and 4°C at the lower refrigeration chamber. The temperature of the laboratory at the time of storage was 28°C and this was taken as the ambient temperature.

iii. Sensory analysis:

The method adopted was a conventional method used by Kremdorf et al,(1979). The characteristics features of the fish such as the colour of the eyes, skin and gills were observed. The odour and texture of the tissue and development of the slime on the surface were also observed as the storage period increased.

iv. Determination of pH:

pH of the fish was determined by the method of Waller (1980). 10g of the fish sample were homogenised with 50mls of distilled water and the pH value of the homogenate was measured by means of a glass electrode pH meter (Munchean 15) that was previously standardised.

v. Microbiological examination:

The bacterial counts on the external surfaces, intestines, gills and tissue were estimated as follows;

a. Skin surface:

A sterilised rectangular wire swab guide measuring 5cm by 2cm was placed on the lateral surface of the fish sample. A sterile cotton wool swab was dipped in 0.10% sterile peptone water and was robbed over the surface of the fish on the area covered by the wire swab guide. The swab was immediately placed in a sterile sample bottle containing 100mls of 0.10% (w/v) peptone water. The bottle was vigorously shaken for 10mins and allowed to stand for 20mins. 10 fold serial dilution of the bacterial suspension in peptone water was prepared in duplicate and viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37°C for 48 hrs as described in Slaby et al, 1981.

b. Intestines, Gills and Tissues:

10g of the fish sample was dissected out, blended and mixed properly in a mortar. It was ascetically transferred to a sample bottle containing 90mls of 0.1% sterile peptone water. The bottle was closed and shaken thoroughly for 10 mins. and

allowed to stand for 20mins, after which a 10 fold serial dilution was carried out in duplicates and viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37^0C for 48 hrs as described in Slaby et al, 1981.

c. Identification of Microorganisms:

Morphological characteristics of the various bacterial isolates were noted in the agar plates and after staining reactions and series of biochemical tests, individual microbial species were identified as described in Slaby et al, 1981.

vi. Determination of Trimethyl Amine-Nitrogen (TMA-N)

Trimethyl-amine nitrogen(TMA-N) was determined by a slight modification of Conway Microdiffusion Method (1968). 25g of the fish sample was chopped and mixed thoroughly with 75 mls. distilled water in a 250 ml. beaker. The pH was adjusted to 5.2 by addition of few drops of 2N HCL, this was followed by heating at 70^oC and cooling to room temperature. After cooling, the sample was filtered into a conical flask with the aid of whatman No. 1 Filter paper. 2mls. of 0.025N HCL was transferred to the central compartment of the micro diffusion dish with the aid of a pipette, this was followed by the addition of 2mls. of the extract and 0.5mls. of 35% formaldehyde with 1 mls. of saturated K_2C0_3 solution into the outer ring. The dish was covered immediately with a glass plate and the set up was left at room temperature for 24hrs. After this, the HCL in the inner compartment was titrated with 0.025N NaOH using 2-3 drops of methyl red/methylene blue indicator. The result was expressed in mg, TMA-N/100g of fish as described in Conway (1968).

vii. Determination of Total Volatile Bases (TVB-N)

Total Volatile Bases (TVB-N) was determined by a slight modification of Conway Microdiffusion Method (1968). 25g of the fish sample was chopped and mixed thoroughly with 75 mls. distilled water in a 250 ml. beaker . The pH was adjusted to 5.2 by addition of few drops of 2N HCL, this was followed by heating at 70^oC and cooling to room temperature. After cooling, the sample was filtered into a conical flask with the aid of whatman No. 1 Filter paper. 2mls. of 0.025N HCL was transferred to the central compartment of the micro diffusion dish with the aid of a pipette, this was followed by the addition of 2mls. of the extract and 1 mls. of saturated K_2C0_3 solution into the outer ring. The dish was covered immediately with a glass plate and the set up was left at room temperature for 24hrs. After this, the HCL in the inner compartment was titrated with 0.025N NaOH using 2-3 drops of methyl red/methylene blue indicator. The result was expressed in mg, TVB-N/100g of fish as described in Conway (1968).

The Statistical Measure used in the quantification of result is Linear Correlation Correlation coefficient (r)

$$r = \frac{\sum (x - \bar{x})\ (y - \bar{y})}{\sqrt{\sum (x - \bar{x})^2 (y - \bar{y})^2}}$$

y = Tma/Tvb (mg/100g),

x = Log TVC (total viable counts) (cfu/g)

$\bar{x}$ = Mean of x, $\bar{y}$ = Mean of y.

Strong and perfect correlation should be near 1 or -1 (0.999 or -0.999)

3. Results:

Population densities and varieties of bacterial isolates from the fish sample under different storage conditions

The population densities of various bacterial isolates from the fish sample were monitored at different storage temperatures i.e. 28^0C, 4^0C and -5^0C and the experiment was terminated when the spoilage pattern exceeded the proposed international limits for the evaluation of the shelf life of fish and fishery products. Bacterial organisms were isolated from different parts of the fish body such as surface skin, gills, intestines and tissue. At ambient temperature, the experiment was monitored for 18hr.s as shown in table 1. Refrigeration storage (4^0C) was monitored for 12 days while frozen state storage(-5^0C) was monitored for 5 weeks, the results were shown in tables 2 and 3 respectively. It was generally observed that both the population and types of bacterial flora increased as spoilage progresses except at frozen storage where the reverse was the case.

Values of Trimethylamine (TMA-N), Total volatile bases (TVB-N) and pH recorded at different storage temperatures

In contrast to the microbiological examination where different organs and tissues of the fish sample were used for the evaluation, only intact tissue of the fish was used for the determination of TMA-N, TVB-N and Ph. and the experiment was monitored for 24 hrs. at ambient temperature storage as shown in table 4, 15 days at refrigeration storage as shown in table 5 and 5 weeks at frozen storage as shown in table 6. As in microbiological studies, the experiment was terminated when the TMA-N and TVB-N values exceeded the proposed international limits for the evaluation of fish and fishery products.

Table 1: Types and Population Densities of Bacteria isolated from the Fish sample (*Pseudotolithus elongatus*) during storage at ambient temperature at 28°C for 18hrs

Storage Time (Hrs.)	Bacteria Isolate	Total Viable Counts (TVC) Cfu/g or Cfu/cm^2
0		
External Surface	*Micrococcus sp., Bacillus sp., Pseudomonas* sp., *Acinetobacter* sp., *Lactobacillus* sp, *Micrococcus* sp, *Corynebacterium* sp,	2.2 x 10^3 Cfu/cm^2
Tissue	*Bacillus, Micrococcus* sp.	7 x 10^2 Cfu/g
Gills	*Flavobacterium* sp, *Bacillus* sp, *Micrococcus* sp	3.8 x 10^5 Cfu/g
Intestine	*Vibrio sp*., *Flavobacterium* sp., *Escherichia* sp., *Bacillus* sp., *Micrococcus* sp. Corynebacterium sp.	6.3 x 10^5 Cfu/g
6		
External Surface	*Peoteus sp., Corynebacterium* sp, *Micrococcus* sp., *Pseudomonas* sp., *Flavobacterium* sp.	3.6 x 10^4 Cfu/cm^2
Tissue	*Micrococcus* sp., *Bacillus* sp., *Pseudomonas* sp.	2.5 x 10^3 Cfu/g
Gills	*Micrococcus* sp., *Bacillus* sp., *Vibrio* sp., *Pseudomonas* sp.	3.8 x 10^6 Cfu/g
Intestine	*Proteus sp., Vibrio* sp., *Pseudomonas* sp., *Escherichi*a sp., *Micrococcus, Flavobacterium,*	6.8 x 10^7 Cfu/g
12		
External Surface	*Pseudomonas* sp., *Flavobacterium* sp., *Micrococcus* sp., *Bacillus* sp. *Acinectobacter* sp.	5.8 x 10^5 Cfu/g
Tissue	*Bacillus* sp.,*Pseudomonas* sp., *Micrococcus* sp.	8.2 x 10^4 Cfu/g
Gills	*Escherichia* sp., *Vibrio* sp., *Pseudomonas* sp.,*Bacillus* sp., *Pseudomonas* sp.	6.2 x 10^7 Cfu/g
Intestine	*Acinetobacter* sp., *Pseudomonas* sp., *Corynebacterium* sp., *Bacillus* sp.Proteus sp.	3.1 x 10^8 Cfu/g
18		
External Surface	*Vibrio* sp., *Escherichia* sp., *Pseudomonas* sp., *Micrococcus* sp., *Bacillus* sp.Proteus sp.,	4.2 x 10^6 Cfu/cm^2
Tissue	*Pseudomonas sp., Vibrio* sp., *Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp., *Corynebacterium* sp.	4.2 x 10^6 Cfu/g
Gills	*Pseudomonas* sp., *Micrococcus* sp., *Vibrio* sp.,	5.6 x 10^8 Cfu/g
Intestine	*Flavobacterium* sp., *Escherichia* sp., *Bacillus* sp. *Proteus, Vibrio* sp., *Corynebacterium.*	2.9 x 10^9 Cfu/g

Table 2: Types and Population Densities of Bacteria isolated from the Fish sample (*Pseudotolithus elongatus*) during storage at a temperature of 4°C for 12 days

Storage Time (Days.)	Bacteria Isolate	Total Viable Counts (TVC) Cfu/g or Cfu/cm^2
0		
External Surface	*Micrococcus sp.*, *Bacillus sp.*, *Pseudomonas* sp., *Acinetobacter* sp., *Lactobacillus* sp, *Corynebacterium* sp,	2.2 x 10^3 Cfu/cm^2
Tissue	*Bacillus*, *Micrococcus* sp.	7 x 10^2 Cfu/g
Gills	*Flavobacterium* sp, *Bacillus* sp, *Micrococcus* sp	3.8 x 10^5 Cfu/g
Intestine	*Vibrio sp.*, *Flavobacterium* sp., *Escherichia* sp., *Bacillus* sp., *Micrococcus* sp. Corynebacterium sp.	6.3 x 10^5 Cfu/g
3		
External Surface	*Pseudomonas* sp.,*Micrococcus* sp., Corynebacteria sp.,	4.2 x 10^3 Cfu/cm^2
Tissue	*Micrococcus* sp., *Pseudomonas* sp., Bacillus sp.,	8.7 x 10^3 Cfu/g
Gills	*Corynebacterium sp.*, *Pseudomonas* sp., *Escherichi*a sp., *Micrococcus sp.*	4.8 x 10^5 Cfu/g
Intestine	*Escherichia sp.*, *Flavobacterium sp.*, *Vibrio sp.*,*Pseudomonas sp.*, *Bacillus sp.*,	3.8 x 10^6 Cfu/g
6		
External Surface	*Bacillus sp.*, *Pseudomonas* sp., *Flavobacterium* sp., *Micrococcus* sp.,	6.8 x 10^3 Cfu/g
Tissue	*Pseudomonas* sp., *Micrococcus* sp.	3.6 x 10^4 Cfu/g
Gills	*Escherichia* sp., *Vibrio* sp., *Pseudomonas* sp.,*Bacillus* sp., *Pseudomonas* sp.	2.2 x 10^6 Cfu/g
Intestine	*Vibrio* sp., *Pseudomonas* sp., *Bacillus* sp., Proteus sp., Escherichia sp. *Micrococcus* sp., *Corynebacterium* sp.	2.5 x 10^7 Cfu/g
9		
External Surface	Pseudomonas sp., Micrococcus sp., Flavobacterium sp.	4.5 x 10^4 Cfu/cm^2
Tissue	*Micrococcus* sp., *Pseudomonas* sp,.	4.8 x 10^4 Cfu/g
Gills	*Vibrio sp.*,*Pseudomonas sp.*, *Micrococcus sp*.	6.2 x 10^6 Cfu/g
Intestine	*Escherichia* sp., *Pseudomonas* sp., *Vibrio* sp.,Proteus sp.	5.2 x 10^8 Cfu/g
12		
External Surface	*Pseudomonas* sp., *Micrococcus* sp., *Acinetobacter* sp., *Flavobacterium* sp.,	5.2 x 10^5 Cfu/cm^2
Tissue	*Pseudomonas* sp., *Micrococcus* sp., *Flavobacterium* sp.	3.6 x 10^6 Cfu/g
Gills	*Micrococcus* sp., *Pseudomonas* sp.	3.8 x 10^7 Cfu/g
Intestine	*Proteus sp.*, *Escherichia* sp., *Pseudomonas* sp., *Vibrio* sp.,	4.2 x 10^9 Cfu/g

Table 3: Types and Population Densities of Bacteria isolated from the Fish sample (*Pseudotolithus elongatus*) during storage at a temperature of -5°C for 5 Weeks

Storage Time (Weeks)	Bacteria Isolate	Total Viable Counts (TVC) Cfu/g or Cfu/cm^2
0		
External Surface	*Micrococcus sp.*, *Bacillus sp.*, *Pseudomonas* sp., *Acinetobacter* sp., *Lactobacillus* sp, *Corynebacterium* sp,	2.2 x 10^3 Cfu/cm^2
Tissue	*Bacillus*, *Micrococcus* sp.	7 x 10^2 Cfu/g
Gills	*Flavobacterium* sp, *Bacillus* sp, *Micrococcus* sp	3.8 x 10^5 Cfu/g
Intestine	*Vibrio sp.*, *Flavobacterium* sp., *Escherichia* sp., *Bacillus* sp., *Micrococcus* sp. Corynebacterium sp.	6.3 x 10^5 Cfu/g
1		
External Surface	*Pseudomonas* sp., Acinetobacter sp.	2.5 x 10^3 Cfu/cm^2
Tissue	*Micrococcus* sp., *Pseudomonas* sp.	2.3 x 10^3 Cfu/g
Gills	*Micrococcus* sp., *Proteus sp.*	2.4 x 10^4 Cfu/g
Intestine	*Escherichia* sp., *Vibrio sp.*	3.8 x 10^{4}Cfu/g
2		
External Surface	*Pseudomonas* sp.	4.7 x 10^3 Cfu/cm^2
Tissue	*Pseudomonas* sp., *Micrococcus* sp.	3.6 x 10^3 Cfu/g
Gills	*Micrococcus* sp., Proteus sp.	2.8 x 10^4 Cfu/g
Intestine	*Pseudomonas* sp., *Flavobacterium* sp.,	3.2 x 10^4 Cfu/g
3		
External Surface	*Pseudomonas* sp., *Acinetobacter* sp.	2.2 x 10^3 Cfu/cm^2
Tissue	*Pseudomonas* sp.	3.5 x 10^3 Cfu/g
Gills	*Pseudomonas* sp.,	1.8 x 10^4 Cfu/g
Intestine	*Pseudomonas* sp., *Vibrio* sp.	3.2 x 10^4 Cfu/g
4		
External Surface	*Pseudomonas* sp.	2.5 x 10^3 Cfu/cm^2
Tissue	*Pseudomonas* sp.	1.7 x 10^4 Cfu/g
Gills	*Pseudomonas* sp.	2.8 x 10^4 Cfu/g
Intestine	*Pseudomonas* sp., *Vibrio* sp.	4.8 x 10^4 Cfu/g
5		
External Surface	*Pseudomonas* sp.	1.5 x 10^3 Cfu/cm^2
Tissue	*Pseudomonas* sp.	4.2 x 10^4 Cfu/g
Gills	*Flavobacterium* sp.,	1.7 x 10^4 Cfu/g
Intestine	*Pseudomonas* sp., *Vibrio* sp.	2.8 x 10^4 Cfu/g

Table 4: Values of Trimethylamine (TMA-N), Total volatile bases (TVB-N) and pH recorded at ambient temperature (28°C)

Storage time (Hrs.)	TMA-N(mg/100g)	TVB-N (mg/100g)	pH
0	1.40	19.90	6.6
6	3.40	26	6.9
12	5.20	24.80	7.4
18	12.20	77.50	7.2
24	20.40	104.90	8.1

Table 5: Values of Trimethylamine (TMA-N), Total volatile bases (TVB-N) and pH recorded at refrigeration temperature (4°C)

Storage Time (Days)	TMA-N (mg/100g)	TVB-N (mg/100g)	pH
0	1.40	19.90	6.6
3	5.40	28.10	6.7
6	5.60	32.90	6.8
9	8.80	51.40	7.2
12	13.70	99.40	7.2
15	16.80	95.30	7.6

Table 6: Values of Trimethylamine (TMA-N), Total volatile bases (TVB-N) and pH recorded at Freezing temperature (-5°C)

Storage Time (Weeks)	TMA-N (mg/100g)	TVB-N (mg/100g)	pH
0	1.40	19.90	6.6
1	1.20	16.30	6.7
2	6.10	21.40	6.9
3	7.50	30.80	7.1
4	7.80	34.30	7.1
5	11.50	37.70	7.2

4. Discussion:

The quality assessment of a typical Nigerian marine Fish commonly known as Croaker (*Pseudotolithus elongatus*) was based mainly on sensory, microbiological and chemical method of evaluation. During sensory evaluation, the colour of the fish skin, texture of the flesh, the colour of the eyes and the gills and the development of offensive odour were observed during storage. The criteria for rejection was based on the development of strong ammonical and offensive odours, softening of the tissues, discolouration of the skin and very high microbial counts of the Fish tissue which correlated with high values of Trimethyl amine nitrogen (TMA-N) and Total volatile bases (TVB-N) and the change of the Fish pH to alkalinity.

The bacterial load of the Fish sample before storage and subsequent analysis was 2.2 x 10^3 cfu/cm^2 on the skin surface and 7 x 10^2 cfu/g on the tissue. The Gills and the intestine of the freshly caught Fish sample harboured a bacterial load of 3.8 x 10^5 and 6.3 x 10^5 cfu/g respectively. The relatively high bacterial load and varieties of bacterial isolates from the Fish sample was an indication of the method of thrawling adopted by NIOMR which must have exposed the Fish sample to the high bacterial load of the disturbed bottom sediment where Fish nets were trawled along the bottom sediment for long periods.

The handling procedure of Fishes in tropical countries like Nigeria stimulated the storage trial at ambient temperature. The common handling practice involves partial icing during fishing followed by marketing at ambient temperature.

The shelf life of the Fish sample (Croaker) at ambient temperature was predicted to be 12 hrs. At this time, the general appearance of the Fish was still sensorily acceptable. The bacterial load of the Fish tissue was 8.2 x 10^4 cfu/g and values of TMA-N and TVB-N at this time were 5.20 and 24.80 mg/100g respectively. These values fall within the acceptable range for international trade (ICMSF 1978, Martins et al, 1978; Gorczyca and Pohlen, 1985 and Connel, 1995). Some of the definitive attempts that were made to study sea food spoilage at ambient temperature include the work of Reilly et al, 1985 where it was observed that brackish water prawns spoil within 16hrs at ambient temperature. Gorczyca and Pohlen, 1985 observed that Trout, Beam and Mullet spoil within 13hrs. at ambient temperature. Ola and Oladipo (2004) have also predicted the shelf life of a similar Nigerian marine Fish, Croaker (*Pseudotolithus senegaliensis*) to be 12hrs, at ambient temperature using sensory, microbiological and chemical approach.

At refrigeration temperature (4^0C), the storage life of the Fish sample was predicted to be 6 days. At this stage, the fish was still sensorily acceptable. The bacterial load of the Fish tissue was 3.6 x 10^4 cfu/g. TMA-N and TVB-N values recorded at day 6 were 5.60 and 32.90 mg/100g respectively. All these values were within the recommended international standard limits for acceptability (ICMSF 1978, Martins et al, 1978; Gorczyca and Pohlen, 1985 and Connel, 1995). Some authors like Maches (1982) have also observed that Shrimps can keep up to 6 days at refrigeration temperature (5^0C), Huss (1985) have equally observed that the shelf life of most marine Fishes at refrigeration temperature (5^0C) can last up to 5 days depending on the Fish specie, oil level of the Fish, catch area and intrinsic conditions of the Fish.

At freezing temperature (5^0C), the predicted shelf life of the Fish sample was 3 weeks. At this stage, only very slight changes on the external appearance of the Fish was observed. There was no ammonical odour because bacterial spoilage was drastically reduced and tissue softening was minimal but the TMA-N and TVB-N values increased progressively over time though slowly. The bacterial load of the tissue at week 3 was 3.5 x 10^3 cfu/g. TMA-N and TVB-N values were 7.50 and 30.80 mg/100g respectively and within the stated international limits of acceptability. Huss (1995), stated that super chilling at -4^0C and below can effectively prolong the storage life of the fish for up to 5 weeks, this assertion was corroborated by Adams and Moses (2008) and Mhongole (2009).

Generally, there was a strong correlation at ambient and refrigeration temperatures between the total viable counts and the TMA-N and TVB-N values but at frozen temperature, no correlation existed and that explained why the sensory and microbiological analysis did not tally with the TMA-N and TVB-N values at frozen temperature. In a similar study, Hozbor et al, 2006 found a strong correlation between the microbiological changes in sea salmon stored in ice and other quality indices like TMA-N, TVN-N and Histamine but no correlation existed at frozen temperatures (-25°C).

Conclusively, it can be advanced that the shelf life of Croaker *(Pseudotolithus elongatus)* at ambient and refrigeration temperatures were 12hrs, and 6 days respectively and the sensory, microbiological and chemical approach used in the analysis were all in agreement with the recommended international limits for acceptability, however at frozen temperature, no correlation existed between the total viable counts of bacteria and the values of the TMA-N and TVB-N values which were still within the recommended acceptability limits. This is understandable because at frozen temperature, microbial spoilage is very unlikely because very few microbial species can grow at such temperatures, Fish spoilage however can be due to biochemical and enzymatic changes in Fish tissues and muscles which progresses slowly at such temperatures.

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