Journal of Fisheries International 2 (2): 134-139, 2007

Hypoxanthine Levels Chemical Studies and Bacteria Flora/Count of Alternate Frozen/Thawed Market Simulated Croaker (*Pseudotholithus senegalensis*)

O.A. Oyelese
Department of Wildlife and Fisheries Management, University of Ibadan, Ibadan, Nigeria

Abstract: The determination of the hypoxanthine levels chemical studies and bacteria flora/ count of alternate frozen/thawed market simulated croaker (*Psendotholithus senegalensis*) was carried out weekly for 12weeks under cold storage conditions at -4°C. Twenty two pieces of Croaker (*Psendotholithus senegalensis*) caught within the Nigerian Coastal waters with an average weight of 270 g were removed fortnightly allowed to thaw and exposed for 12 h before taken them for analysis. While organoleptic assessment and biochemical parameters (Hypoxanthine levels (Hx), Trimethylamine (TMA) Peroxide Value (PV) and Free Fatty Acid (FFA) were carried out fortnightly, however microbial assessment (bacteria count and identification) were assessed every 4weeks (monthly). Also initial and final proximate analysis were carried out. The cold storage medium does not totally halt spoilage, this is because hypoxanthine levels increased with storage length just as the bitter taste becomes more pronounced by the end of the 12weeks, this is further confirmed by the organoleptic results which also showed a progressive increase in their values. The biochemical test revealed that all parameters had a consistence increase in their values as the length of storage period increase, significant increase ($p<0.05$) was recorded for (PV, TMA, FFA and Hx) with strong positive correlation (R=0.98, 0.99, 0.96 and 0.96, respectively) with length of storage period. The microbial count and identification results shows that 7 bacteria species have invaded the samples, in which *Micrococcus acidophilus* is the only bacteria with the lowest count of 1.60×10^4 cfu g^{-1} (and also showed it presence in the 12th week) and also out of the 7 bacteria identified 4 were prominent which include *Lactococcus acidophilus* (occurred from 0-12th week) *Clostridium welchii* (4-12th week) *Bacillus substilis* (8-12th week) and *Proteus morganii* (8-12th weeks) had the highest number of occurrence with (5.58, 4.29, 3.37and 3.18×10^4 cfu g^{-1}). Microbial build up rises with increase in the length of storage period, this is because the Total Viable Count (TVC) increase from 0 week (1.64×10^4 cfu g^{-1}) to(10.5×10^4 cfu g^{-1}) in the 12 week. However the limit of acceptability for Croaker (*Pseudotolithus species*) under cold storage at -4°C is 8 weeks (2months) since values of all measured parameters increased drastically and sharply at the end of the 8th week.

Key words: Croaker (*Pseudotolithus senegalensis*), hypoxanthine levels, organoleptic/chemical/bacteria assessment, frozen/thawed, market simulated, shelf life

INTRODUCTION

Hypoxanthine is a normal constituent of fish flesh though present in very low concentrations in life fish. It is the end product of a series of enzymatic reaction going on in the fish flesh which gives the characteristic bitter after taste to cooked stale spoiling fish when eaten, as shown.

ATP, ADP, AMP, IMP, Inosine, Hx. Adenosine Triphosphate (ATP) is broken down into Adenosine Diphosphate (ADP) though the loss of one phosphate energy bond. This is further broken down to Adenosine Monophosphate (AMP) and eventually to Inosine Monophosphate (IMP).

This is further broken down to Inosine and the end product of this series of chemical reaction is the production and accumulation of Hypoxarthine (Hx) in the dead fish flesh as a result of nucleotide degradation. This is further explained as follows DMAB breaks ATP into ADP, AMP and IMP is removed when placed in the water bath at 20°c then it is left with Inosine, which is the substance that cause colour development (discolouration) in spoiling fish. The amount of the colour developed is measured by spectrometer. Hypoxanthine unlike TMA and TVB increases in most fish species soon after death and in the early days of storage (Howgate, 1982; Burt, 1977; Howgate *et al*., 1972; Howgate, 1965). Generally, the measurement of Hypoxanthine (Hx) content is a better index of freashness and gives a better indication of spoilage over a wide range of qualities Adenosine Triphosphate (ATP) is degraded via Inosine to hypoxanthine mainly due to a utolytic processes but in

the latter phases, bacterial action may also be involved. Measurement of Hypoxanthine (Hx) should therefore, give a good indication of early post mortein changes in fish. Fish is one of the most perishable goods of all the foods quality foods protein sources even though it is important in human diet.

This perishable nature is due to the production of high content of low molecular weight metabolites for microbial nutrition. Also due to its total bodyweight and low connective tissue which makes it highly susceptible to bacterial attack. The high temperature of the tropics makes fish spoil with in twelve h of death. Its perishability is also closely related to the structure and chemical composition. Fish in a good condition which is made up of five components namely protein, carbohydrates, ash, moisture and lipids which are later affected by bacteria and oxygen, from the moment the fish dies. A high water content in fish makes Fish liable to high microbial attack; since this forms a favourable medium for rapid bacteria growth as reported by Stansby (1992). Just after the death of fish, the protein components which are polymers of amino-acids are initially decomposed by proteases enzymes. This increase the content of the non-protein nitrogen (Indole, Skatole, TMA and DMA) and makes fish flesh more alkaline and therefore deterioration sets in quickly. These compounds are responsible for offensive smell, rancid odour and off flavours.

Croakers (*Pseudotolithus* sp.) are primarily marine fish species but also occurs seasonally in brackish water areas. Most of the species inhabit sandy and muddy bottoms in coastal areas with large river flows. *Pseudotolithus* species belongs to the family Sciaenidae. *Pseudotholitus senegalensis* (cassava croaker) is found along the West African Coast between Morocco and Angola but is rare mouth of Senegal. It is found along with other species like *Pseudotholitus* elongates in muddy, sandy and rocky bottoms from the shoreline to 70 m depth. The smaller and younger ones prefer shallow waters and move to mid waters when bottom temperature falls below 18°C. The two species are jointly harvested by artisanal and industrial fisheries using illnots, beach seing; longlines and bottom trawls. It is common practice that stale and spoilt fish are sold in the open market and the mode of handling and the preservation given to this fish determines the final quality of the fish to the consumers. This study tends to simulate the market situation before final sale, which allows the ice to thaw for h in the open market and most times the fish that are not sold are returned daily to the cold store. In most cases the freshness of the fish due to constant thawing and exposure leaves the fish with a bitter after taste when cooked and eaten which is due to accumulation of Hypoxanthine (Hx).

It is therefore of interest to probe more on the hypoxanthine levels, along with order chemical and bacteria assessment studies with length of cold storage. The objectives of this study are:

- To determine the hypoxanthine levels in *Pseudotolithus senegalensis* under cold storage with storage length.
- To determine the relative rate of spoilage in relation to storage length using some biochemical parameters such as Peroxide Value (PV). Trimethylamine (TMA), Free Fatty Acid (FFA), organoleptic assessment and bacteria flora/count.
- To establish the limit of acceptability of this fish stored under frozen condition.

MATERIALS AND METHODS

Twenty two pieces of Croaker (*Pseudotolithus senegalensis*) caught within the Nigerian Coastal waters with an average weight of 270 g were stored in a cold room at -4°C for an experimental period of 12 weeks.

Two pieces were removed fortnightly allowed to thaw and exposed for 12 h to simulate the open market situation (where fish are left for prospective buyers) before taking them for analysis. The experiments were in four parts:-

- Proximate Analysis-Initial and final proximate analysis were carried out at the beginning of the 1st week and the 12th week, respectively.
- Biochemical Assessment,
- Hypoxanthine (Hx) levels
- Peroxide Value (PV)
- Trimethylamine (TMA)
- Free Fatty Acid (FFA) were carried out forthnightly.
- Organoleptic assessment for both cooked and uncooked Croaker were also carried out fortnightly.
- Microbiological analysis for identification of bacteria flora and count was also carried out every weeks.

Proximate analysis of *Pseudotolithus senegalensis*: The proximate analysis was determined for crude protein, moisture, fat and crude fibre on dry matter basis according to A.O.A.C. (1990) methods procedures.

Samples of *Pseudotolithus senegalensis* were analysed at the beginning of the first week before storage to form the initial proximate analysis, while the final proximate composition was determined at the end of the 12th week experimental period.

Biochemical assessment: Also initial baseline data were obtained for all the biochemical parameters initially on the

1st day before the commencement of the experiment, while samples were subsequently withdrawn every 14 days (2 weeks) for analysis.

Determination of hypoxanthine: Two g of crushed fish sample was weighed into a 250 mL beaker, 1 g of active carbon 100 mL of distilled water and 5 mL of carez solution 1 and 2 were added and mixed for 30 min. The mixture was filtered through a whatman No.2 fitter paper. Five mL of clear colourless filtrate was pipetted into 15 mL test tube, 5 mL of 4-DMAB solution added mixed and placed in the water bath at 20°C The absorbance of the mixture was taken after colour development on a spectronic 2ID spectrophotometer at a wavelength of 460 nm. Standard hypoxanthine of range 2-10 ppm was also treated as sample and absorbance taken at the same wavelength.

$$\text{Conc. of hypoxanthine in mg } 100^{-1} \text{ gm fish} = \frac{\text{Absorbance of sample} \times \text{gradient distillation factor of standard}}{\text{Weight of sample}}$$

As reported by Burt (1977).

Peroxide Value (PV) test: Two gram of crushed fish sample was weighed into 250 mL beaker. Twenty milli liters of chloroform and 10 mLs of Glacial acetic acid were added to the fish sample in the beaker and mixed (PV as carried out forth-nightly).

The mixture was filtered into 250 mL conical flask. One mililiter of 5% aqueous saturated potassium iodide (KI) solution was added and shaken thoroughly. The homogenous mixture was placed on the hot plate to boil for 30 sec. Add 25 mL distilled water, shake add 1 mL 1% starch and titrate the hot mixture against 0.002 M$Na_2S_2O_3$.

A blank determination was also carried out at the same time. Peroxide value is the Nos of mls of $Na_2S_2O_3$(0.002 m) used for the titration in Meq Kg^{-1}.

$$\text{PV (Meq kg}^{-1}) = \frac{\text{(Titre value of sample taken-Titre value of blank)} \times \text{MNa2S2O3} \times 103}{\text{Weight of the sample}}$$

As reported by Burt *et al*. (1976).

Trimethylamine acid test (TMA): This test was carried out using a semi-microdistillation procedure (Burt *et al*., 1976).

Hundred gram of crushed fish sample was weighted into a 500 mL beaker 300 mL of 5% Tetra-Chloroacetic Acid (TCA) was added, macerated and homogenized to obtain a clear extract. Five mL of the extract was pipetted into the Markham distillation apparatus, 5 mL of 2 MNaOH was added and steam distilled into a 100 mL conical flask containing 15 mL of 0.01 MHCL.1 mL of 1% resolic acid indicator added and titrated against 0.01 m NaOH to obtain a pale pink colour and point to get $T.V_1$, add 1 mL of 16% formaldehyde (neutralised) to the mixture n the titration flask to liberate excess acid. Titrate the excess acid with 0.01 m NaOH to obtain $T.V_2$.

$$\text{Trimethylamine} = \frac{14(300+ \text{weight of sample taken}) \times \text{T.V2}}{\text{Nitrogen } 500}$$

$T.V_2$=Titre value of the liberated or excess acid.

Free fatty acid determination: Two gram of crushed fish sample was dissolved in a 50 mL mental solvent of diethyl ether and alcohol (1:1). The mixture was thoroughly shaken to dissolve the fish content. The mixture was then titrated against 0.I M NaoH using 1% phenolphthalein as indicator to obtain a faint pink colour at the end point.

$$\text{\% free fatty acid} = \frac{\text{Titre value of sample} \times 4.0 \times 1}{\text{Weight of sample used 2}}$$

Organoleptic assessment: This assessment was based on the scoring system which involve measurement of certain parameters on graded scores. Therefore a 5 man panel was briefly trained on the organoleptic or sensory assessment. Sample from the stored fish were then accessed, with a scoring system scales ranging from 1 to 6 based on the determination characteristics (Emokpae, 1979).

For uncooked fish, a whole round fish was placed on a clean table for grading. Parameters employed by the judges are as follows:-

- Appearance or external characteristics which includes pigmentation of the skin, it means shape of the eye tint, colour of the gills, rigidity of the abdominal wall and colour of the flesh.
- Texture refers to the degree of loss of elasticity of the flesh.
- Odour
- Colour
- Taste for the cooked fish.

The fish sample were filleted and steamed for 25 min and presented to the taste panel on plates. The panelists were asked to rinse their mouth before tasting, so as to avoid any bias in the result.

The scores were based on the following

- Taste
- Flavour.

Microbiological analysis: One gram of the ground fish sample was dissolved into sterile peptone water. Specimen bottles and sterile test tubes were further sterilized before putting samples into them.

Pipette 9 mL of peptone water into each of the test tube, add 1 mL of test sample to the first tube via 1 mL of the first dilution using another sterilized pipette from the first dilution and add labelled as 10^{-2}. another third pipette is used to prepare 1 mL 100 or 10^{-3} dilution as above. Dilution of 10^{-4}, 10^{-5} etc. can also be done. Similarly depending on the probable bacterial content. Mix the contents of the final dilution tube and discard 1mL into the disinfectant jar. Fifty mL of the last dilution test tube was pippetted into 15 mL of mackonkey Agar and allow to air dry in a pour plate (sterilized plate)

The plates were incubated at 37°C for 24 h and the number of colony developed were counted using Jemway colony counter. Microbial count (TVC) = No of colonies×dilution factor

Data analysis: The statistical tools used to analyse the result obtained are:-

- Correlation analysis of the parameters.
- Simple Linear Regression Analysis, according to Steel and Torrie (1960).

RESULTS

As shown in Table 1, the final proximate composition recorded higher values of crude protein, ether extract (fat) and moisture content compared to the initial proximate composition, but with higher values recorded for crude fibre and ash only in the initial proximate composition.

Table 2 shows that the cooked samples of the Croaker (A_1-A_6) showed progressive deterioration from very good recorded in the initial fresh sample (AB) to fairly satisfactory 5 recorded in the stored sample at the 12th week. It should be noted that samples were withdrawn weekly, thawed and exposed for 12 h before cooking and the above organoleptic assessment were carried out and subsequent biochemical and bacteriological assessment were also carried out.

Table 1: The proximate composition of croaker *(Pseudotolithus senegalensis)*

Parameter (%)	Initial proximate	Final proximate (cold storage at -4°c)
Crude Protein (C.P)	16.32	17.64
Ether extract (fat)	4.28	4.41
Crude Fibre (CF)	5.43	2.41
Moiture content	69.65	72.30
Ash	2.82	2.18
N.F.E	1.50	1.06

Table 2: Organoleptic assessment of (cooked) cold stored (at-4°C) croaker *(Pseudotolithus senegalensis)* length of storage period in weeks

Parameters	0	2	4	6	8	10	12
Samples	AB	A_1	A_2	A_3	A_4	A_5	A_6
Taste	1	2	2	2	3	4	5
Texture	1	1	2	3	3	3	4
Odour	1	2	2	2	3	4	4
Appearance	2	1	2	2	2	3	3
Colour	1	1	2	2	2	3	4

Key, 1- Excellent 4- Satisfactory 7- Poor, 2- Very good 5- Fairly satisfactory AB- Baseline Sample, 3- Good 6- Fair A_1- A_6- Stored Sample

Table 3, shows Hypoxanthine (Hx) values of the cold stored Croaker samples showed a progressive increase from 0 day to 12week which ranged from 25.32-33.84 mg^{-1} 100 g fish. A similar trend was observed for peroxide value with a range 22.50-29.40Meq kg^{-1} fish, Trimethylamine (TMA) value ranging from 24.12-31.20 mg^{-1}100 g fish and Free Fatty Acid (FFA) values ranging from 1.53-2.16%. The Peroxide Values (PV) increase gradually between O and 8 weeks (from 22.50-26.40 meq kg^{-1} fish) and then sharply from (26.40-29.40 meq kg^{-1} fish) between week 8 and 12, showing that spoilage rate increase with length of storage. However a steady increase was observed for TMA from 0-12th week at an almost constant rate from (24.12-31.20 mg^{-1} 100 g fish).

FFA values increases gradually between 0 -10th week with values from (1.53-1.92%) and sharply between 10-12th week with values from (1.92-2.16%), implying that FFA values were very high towards the end of the storage period.

A similar trend was also observed for the Hypoxanthine (Hx) values which increased gradually between 0-10th week from (25.32-30.42 mg^{-1} 100 g fish) and then increase sharply between 10-12th week with values from (30.42-33.84 mg^{-1} 100 g fish). The hypoxanthine levels increased faster towards the 12th week, implying much bitter taste.

Table 4 shows the result of isolates identified on sample under cold storage medium at-4°C every 4 weeks after exposure for 12 h on each sampling occasion. Seven bacteria species were found to have invaded the samples. Out of the 7 bacteria identified the following *Lactococcus acidophilus Clostridium welchii Baccillus substilis* and *Proteus morganii* had the highest number of occurrence with (5.58, 4.29, 3.37 and 3.18×10^4 cfu g^{-1}) as shown in Table 4. It was noticed that microbial build-up rises with increase in the length of storage period. The Total Viable Count (TVC) showed an increase from 0 week (1.64×10^4 cfu g^{-1}) to (10.5×10^4 cfu g^{-1}) in the 12th week with an overall TVC of 23.11×10^4 cfu g^{-1}).

N.B: CFU = Colony Forming Unit.

Table 3: Biochemical assessment of cold stored sample of croaker B1 weekly at- 4°C

	Length of storage period (b1-weekly)						
Parameters determined	0	2	4	6	8	10	12
Peroxide value (Pv) meq kg^{-1}	22.50	24.20	25.40	26.20	26.40	28.20	29.40
Trimethylamine Acid value TMA (TMA) mg^{-1}100mg fish	24.12	25.60	26.50	27.60	28.60	29.90	31.20
Free Fatty Acid (FFA) (%)	1.53	1.58	1.64	1.72	1.84	1.92	2.16
Hypoxanthine (Hx) mg^{-1}100 g fish	25.32	26.42	27.54	28.34	29.26	30.42	33.84

Table 4: Bacteria Flora/Total Viable Count (TVC) of croaker (*pseudotolithus typus)* cold stored at -4°c for a period of 12 weeks

	Monthly (4 weeks interval of bacteria species)				
Parameters determined	0	4	8	12	Total number
Samples of isolated organisms cfu g^{-1} ($x10^4$)	$X10^4AB$	$X10^4A_1$	$X10^4A_2$	$X10^4A_3$	$X10^4AB+A_1+A_2+A_3$
(1) *Bacillus substilis*	-	-	1.27	2.10	3.37
(2) *Clostridium welchii*	-	1.57	1.42	1.30	4.29
(3) *Eschericha coli*	-	0.56	0.82	0.80	2.18
(4) *Lactococcus acidophilus*	1.64	1.31	1.43	1.20	5.58
(5) *Micrococcus acidophilus*	-	-	-	1.60	1.60
(6) *Proteus mangani*	-	-	1.38	1.80	3.18
(7) *Pseudomonas aureginosa*	-	-	1.21	1.70	2.91
Total Viable Count (TVC)	1.64	3.44	7.53	10.50	23.11

Table 5: Correlation coefficient between hypoxanthine, biochemical parameters and Total Viable Count (TVC) for bacteria and length of storage period

Dependent variable	Correlation coefficient 'R'	Decision ($p<0.05$) or ($p>0.05$)
Peroxide Value (PV)	0.985+ve	Significant ($p<0.05$)
Trimethylamine (TMA)	0.997+ve	Significant ($p<0.05$)
Free Fatty Acid (FFA)	0.967+ve	Significant ($p<0.05$)
Hypoxanthine (Hx)	0.965+ve	Significant ($p<0.05$)
Total Viable Count (TVC)	0.986+ve	Significant ($p<0.05$)

Table 6: Simple linear regression analysis/prediction equations for dependent variables

Dependent variables	Independent variable	Prediction equation	R^2	SE
PV	Period of storage in weeks	PV = 22.861+0.530X	0.97	0.432
TMA	"	TMA = 24.266+0.570X	0.99	0.185
FFA	"	FFA = 1.473+0.049X	0.93	0.061
Hx	"	Hx = 24.954+0.630X	0.93	0.815
TVC	"	TVC = 0.248+0.068X	0.97	0.071

Table 5 shows that significant ($p<0.05$) linear positive correlation exist for all parameters measured in the study (PV, TMA, FFA, Hx and Total Viable Count (TVC). This implies spoilage rate increases with storage period; where r = 0.985, 0.997, 0.967, 0.965 and 0.986 for PV, TMA, FFA, Hx and TVC.

Table 6 shows the relationship between PV, TMA, FFA, Hx and TVC with period of storage shows that the prediction equation are linear and can be expressed as

Y = a+bx

Y = dependent variable (PV, TMA, FFA, Hx and TVC)

a, b = beta values, x = period of storage.

PV in Meq kg^{-1}, TMA in mg N 100 g fish, FFA in %, Hx in mg^{-1}100 g fish and TVC in Cfu g^{-1}.

Cfu = Colony forming unit.

DISCUSSION

The result of the proximate composition showed that the samples under cold storage conditions recorded higher values of crude protein, ether extract (fat) and moisture content in their final proximate composition compared with the baseline data sample (initial proximate). Crude protein with an initial value of 16.32% was increased to 17.64% (in the final proximate). Fat with 4.28% was increased to 4.41% and moisture content with 77.64% was increased to 81.56%. While crude fibre and ash values were reduced, crude fibre from 5.43 to 2.41% and ash with 2.82 to 2.18%.

Going by the crude protein, ether extract and ash content values, Croakers are presented to be rich in protein, fats and minerals. Also the closer value recorded under the cold storage conditions shows that the cold medium has an ability to ensure a better keeping and preserving quality of the sample. This is in line with Stansby (1992), Bligh and Dyer (1959) findings.

However, the cold storage medium does not totally halt spoilage, this is because from the study, the hypoaxanthine level increases with storage length just as the bitter taste becomes more pronounced by the end of the 12 weeks. As the hypoxanthine values increased bi-weekly the bitter taste of the fish becomes more prominent has confirmed by the organoleptic results which also showed a progressive increase in their values, also as reported by Emokpae (1979) and Burt (1977).

The biochemical parameters showed a slight but progressive increase in their values as the length of storage period increases. Peroxide Values (PV) from 22.50-26.40 Meq kg^{-1} fish) increases gradually between 0 and 8 weeks and sharply from (26.40-29.40 Meq kg^{-1} fish)

between 8 and 12th week, showing that rancidity which is also a measure of increasing spoilage rate increases with storage length also in line with Oyelese and Adejumu (1998) findings. This is in line with Bligh and Dyer (1959) findings that 20-40 Meq kg^{-1} fish peroxide value is said to correspond with noticeable rancid taste.

Also there is a steady rise in the values of TMA from 0-12 weeks with a range from (24.12-31.20 mg^{-1} 100 g fish). The Free Fatty Acid (FFA) values increases gradually between 0 week and 10th week from (1.53-1.92%) and sharply between week 10 and 12 with values (1.92-2.16%), in line with Oyelese and Adejumu (1998) and Burt *et al.* (1979) findings meaning that the FFA value was very high towards the end of the storage period. The microbial count and identification results showed that 7 bacteria species had invaded the samples. In which the base line data (AB) sample showed *Lactococcus acidophilus* as the only bacteria identified with the lowest bacteria count of 1.64×10^4 Cfu g^{-1} fish out of the 7 bacteria identified. Four bacteria species out of the 7 were prominent which include *Lactococcus acidophilus* which is the only bacteria specie showing its occurrence throughout the experimental from the initial fresh fish 0 week-12th week, *Clostridium welchii* which occurred only in the cold stored samples in the 4-12th week, *Bacillus subtilis* and *Proteus moganii* both bacteria species (i.e., the latter and occurring in the stored samples at the 8 and 12th week. This is also in line with the findings of Howgate (1982).

CONCLUSION

This study showed that hypoxanthine plays a major role in increasing the spoilage rate of *Pseudotolithus senegalensis* as the length of storage period increases, as well as other biochemical parameters (PV, TMA and FFA). Micro-organisms also play a major role in rendering cold stored *Pseudotolithus* species unfit for consumption as the length of storage period increases. The most potent pathogenic bacteria to which Croaker (*Pseudotolithus senegalensis)* is susceptible to is *Lactococcus acidophilus* and it is also the only bacteria specie showing its occurrence throughout the experiment that is both in the initial sample and the cold stored sample (i.e., from 0-12th week) and *Clostridium welchii* only occurred in the cold stored samples from the 4-12th week. All measured parameters in the study indicates a 2 mouth (8 weeks) limit of acceptability for the cold storage of Croaker *(Pseudotolithus senegalensis)* at-4°C.

REFERENCES

Association of Official Analytical Chemists (AOAC), 1990. Official Method Analysis (15th Edn.), AOAC, Washington USA.

Burt, J.R., 1977. Hypoxanthine; A Biochemical Index of Fish Quality Biochemistry.

Burt, J.R., D.M. Gibson, A.C. Jason and H.R. Sanders, 1979. (a) Comparison of Methods of Freshness. Assessment of wet fish II in Ornamental and Chemical Assessment of boxed Experimental Fish. J. Food. Tech., pp: 73-87.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification Can. J. Biochem. Physiol., 37: 911-917.

Emokpae, A.O., 1979. Organoleptic assessment of the quality of fresh fish Occassional paper. Nigeria Institute of Oceanography and Marine Research.

Howgate, P.F., 1982. Quality Assessment and Quality Control In: Fish Handling and Processing. (Eds.), A. Aitken I.M. Mackie, J.H. Merita and M.L. Windsor, Crown Edinburgh, Scotland.

Howgate, P.F. and S.F. Ahmed, 1972. Chemical and bacteriological changes in fish muscle during healing and drying at 30°C. J .Fd. Sci. Agric., pp: 23-25.

Howgate, P.F., 1965. Fish handling and processing. 1st Ed. Minist Agric. Fish. Food. Resh. Center Edunburg, London, pp: 177-183.

Oyelese, O.A. and C.O. Adejumo, 1998. Rancidity Studies and spoilage rate of *Lutianus goreensis* and *pseudotolithus typus.* J. West Afr. Fish., 7: 342-350.

Stansby, M.E., 1992. Proximate Composition of Fish in Fish Nutrition Ed.E Heen and R. Krewzer, London, Fishing News (Book) Ltd for FAO, pp: 55-60.

Steel, R.G.O. and J.H. Torrie, 1960. Principle and Procedure of Statistics. Mcraw Hill Book Company Inc. New York, Toronto and London.