



Qualitative and microbial characteristics of *Parachanna obscura* under cold storage temperature

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Abstract

This research was carried out in order to study the physicochemical changes, microbiological load and sensory changes of *Parachanna obscura* preserved under cold storage temperature (4°C) associated with different processed method (steamed cooked, kiln smoked and raw). *Parachanna obscura* was purchased from fishermen at Eleyele water Dam in Ibadan, Oyo state Nigeria. Changes in chemical composition Trim ethylamine (TMA), Dimethyl amine (DMA), and Thiobarbituric Acid (TBA), Total bacteria count and sensory analysis were observed fortnightly for the period of eight weeks. The results of the sensory evaluation parameters such as Texture, Taste, Colour, Flavour and Overall acceptability as well as the fish sample (S1F, C2F, and R3F), which were examined fortnightly are significantly different in week 2, week 4, and week 8 since (P<0.005), However, in week 6 they are not significantly different P-value (0.13) > α -value (0.05) this indicates that the longer the storage duration the more the quality of the fish are affected at refrigeration temperature. The bacteria colony count in all shows that C2F (Steam cooked) had the highest microbial load, and the organism species of bacteria identified are *B. subtilis*, *S. aureus* and *Salmonella* spp at the end of the experiment, S1F (smoked fish) some species of bacteria were isolated, *B. subtilis* and *Salmonella* spp, *S. epidermis* and also in R3F (fresh fish) the organism species identified was *Salmonella* spp. The physicochemical parameters were significantly different p<0.05 in all the weeks for the three processing methods (kiln smoked, steam cooked and raw). At week 2 cooked *P. obscura* has the significantly (P<0.05) value while raw had the significantly least value (raw, 9.68^c, smoked 10.57^b and cooked 12.50^a DMA (0.87^c, 1.54^b and 1.62^a) TBA (74.40^b, 69.30^b and 102.00^a) cooked was statistically higher than smoked and raw had significantly (P>0.05) similar means values. At week 4 all parameters were significantly (P<0.05) affected by processing method TMA of (raw 9.59^b, smoked 9.12^b and cooked 15.18^a), DMA (0.90^c, 1.55^a and 1.50^b) TBA (73.80^b, 62.10^c and 115.80^a). At week 6 TMA of cooked *P. obscura* was significantly (P<0.05) higher than other (raw 9.43^c, smoked 11.40^b and cooked 14.47^a), DMA of smoked and cooked *P. obscura* were statistically similar and are significantly higher than raw (0.90^c, 1.55^a and 1.50^b), TBA of cooked *P. obscura* are significantly (p<0.05) highest, while raw had statistically (p<0.05) highest values than smoked (73.80^b, 62.10^c and 115.80^a) respectively. The findings of this research revealed that *Parachanna obscura* preserved under refrigeration temperature for the period of eight weeks are fit for human consumption since the total microbial count are within the limit required limit.

Keywords: *Parachanna obscura*, sensory evaluation, physicochemical, microbial load, smoked and steamed cooked, raw and cold storage

1. Introduction

Fish contributes to the survival and health of an individual globally due to its nutritional qualities most especially in the developing nations. Fish provides the major source of proteins, poly-unsaturated fatty acids, vitamin A, vitamin B2, vitamin B6, minerals and micronutrients such as; iron, calcium, iodine, potassium etc. (Davies and Davies 2009) [13]. According to (Davies and Davies 2009) [13] fish has up to 70-84% moisture content; therefore makes it extremely perishable immediately after death. Consumers across the globe increased the consumption of fish and fish product in recent years due to the recognition of their nutritional value. FAO (2016) reported that fisheries and aquaculture as remain an important source of income, employment, food, nutrition and livelihoods for millions of people across the globe. Fish is a source of income which can be used to purchase other additional food items.

But as one kind of perishable and short shelf-life goods, fish deteriorate due to some factors such as microbial metabolism, oxidative reaction and enzymatic activity (Abassi and Ogar, 2012) [1].

Adebowale *et al.* (2008) [3] reported that fresh fish spoilage

can be very rapid after it is caught. The spoilage process called *Rigor mortis* will start within 12 hours of their catch in the high ambient temperatures of the tropics (Berkel *et al.*, 2004) [10]. *Rigor mortis* is stiffening of the joints and muscles of a fish body few hours after death,

Fish is generally a healthy food source and can be safely eaten in most cases which have been the most selected ingredient on the global menu, an important factor in the global ecological balance, an important basis for livelihood (Abbas *et al.*, 2009) [2]. Fish has no religiosity taboo or cultural restrictions compare to pork meat which makes it more advantageous than others (Adedeji and Okocha, 2011) [4]. Malnutrition has become a trending problem in many countries most especially in Africa. It was estimated that 47 million children under five years old are stunted in sub-Saharan Africa whereas in eastern and southern Africa the figure stands at 24 million. Micronutrient deficiencies of vitamin A, iron and iodine are also of public health concern in the whole African region. Their consequences include nutritional blindness, poor learning capabilities, poor growth and increased morbidity and mortality rates. Development and agricultural programmes including fisheries and

aquaculture which mainstream nutrition issues can go a long way in alleviating the problem of malnutrition in this part of the world as well as in other countries. Fish has been identified as a preferred source of animal protein containing essential minerals and a balanced amino acid profile which obviously promote healthy human growth (Ekunwe and Emokaro, 2009) [14] and the demand for fish is on the increase due to its health benefits of eating fish and due to increase in population (Engvang and Nielsen, 2001) [15]. So there is a need to investigate the physicochemical changes, sensory evaluation and microbial activities responsible for deterioration because of the societal health impact. The specific objective of the study is to evaluate the physicochemical changes; Trimethylamine (TMA), Thiobarbituric Acid (TBA) and Dimethylamine, know the sensory evaluation (Texture, Taste, Colour, Flavour, and Overall Acceptability) of the fishes under investigation and

to examine the microbial activities responsible for deterioration.

2. Methodology

The study area

The samples of fish were collected at Eleyele water dam in Ibadan, Oyo State, Nigeria. Eleyele dam (fig. 1) is located in a North-Eastern part of Ibadan, southwestern Nigeria within longitude E030 511 0 II and E030 521 37II and latitude N070 251 14II and N070 261 40II. Eleyele catchment lies between longitude E030 481 04II and E030 551 17II and latitude N070 211 49II and N070 291 55II and it also comprises of areas such as Apete, Awotan, U.I, Akere, Ojo, Atalapa, Gege, Odo-ona. The dam is a modified natural riverine wetland type with an area of about 1.290 km2 and a catchment area of 10.303 km2 (Olaosebikan and Raji, 1998).

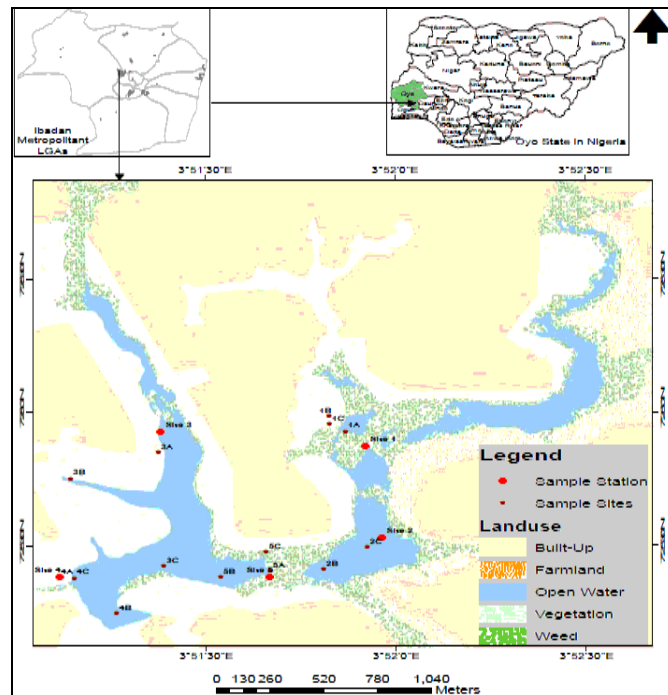


Fig 1: Map showing the Eleyele reservoir

Sample collection and treatment

Three (3) of the same sample species of Freshwater fish *Parachanna obscura* (Snake head), was purchased from Fisherman in Eleyele Ibadan.

The three samples were degutted immediately limiting access of most spoilage bacteria growth. The visceral residues are washed in a tube with ice and clean freshwater.

The fish samples were transported in ice to the Microbiology Research Laboratory (MRL) of The Federal Polytechnic Ado Ekiti for quality characteristics analysis. Each of the fish represents a sample of an equal portion of about 500g each and labeled appropriately thus:

- R3F= Raw
- C2F= steamed cooked
- S1F=kiln smoked

Processing procedures

Raw – The first portion was in the raw form used as a control.

Steamed cooked – The second portion was wrapped in

aluminum foil and place it on a wire rack suspended over boiling water in an aluminum pot. Thereafter, steam cooked for 15 minutes without adding salt, it was allowed to cool and was thereafter stored in refrigeration.

Kiln Smoked – the third portion was kiln smoked using charcoal as a source of heat. The fish were smoked for less than 12 hours with smoking temperatures ranged between 80°C and 105°C. The dried samples were wrapped in foil nylon. Thereafter, it was stored under refrigerator temperature.

Fortnightly experimental samples were taken randomly which represented the whole body of the fish. Thereafter, the samples were chopped with skin and finally ground in mortal and pestle to make homogenous.

Determination of shelf life

The determination of the shelf life of smoked, cooked and raw fish was determined using the bacteriological load, Physico Chemical parameters such as Dimethylamine (DMA), Thiobarbituric Acid (TBB), Trimethylamine

(TMA) and sensory evaluation assessment, such as (Texture, Taste, Colour, Flavour and Overall Acceptability) as described below. The shelf life was determined by preserving each of the differently processed fish samples into aluminum foil, the raw and cooked are stored under refrigeration temperature and the smoked fish is also stored in refrigeration temperature conditions for the period of 8 weeks. The attributes and conditions of the stored fish were assessed fortnightly.

Bacteriological Assessment

Sample preparation was made using the method described by (Obi and Krakowiaka, 1983) [17].

First and foremost, the Petridis and test tube were sterilized in an oven. Thereafter, some part of the fish body were chopped in mortar and pestle and swab stick was used to swab the fish body and inserted into the first test tube containing 9 ml of distilled water as a stock, and five other test tubes also containing 9 ml of distilled water were arranged serially in the test tube rack. 1 ml. of the stock was collected using a pipette to the first test tube and from the first test tube to the second test tube up to the fifth test tube respectively. 1 ml. was taken from each test tube into a sterilized petridish in duplicate. All plates were incubated at a temperature of 37°C for a period of 24 hrs, before colony counting and isolation procedures was carried out.

Preparation of nutrient agar

Nutrient agar was prepared according to the manufacturer description by weighing 28g and dissolved in 1 litre of distilled water. The dissolved nutrient agar was then autoclaved at a temperature of 121°C for 15 minutes. The media was allowed to cool down and pour into each of the petridishes containing 1 ml. of the diluents. It was then allowed to solidify and incubate at temperature of 37°C for 24 hrs.

Identification and Characterization of the Isolates

Identification of the isolates was carried out based on the method described by Olaosebikan and Raji, (1989)

Sensory Evaluation assessments

A fifteen (15) semi-trained panelists were randomly selected consisting of HND II students of Food Science and Technology, The Federal Polytechnic, Ado Ekiti. The parameters such as Texture, Taste, Colour, Flavour and Overall Acceptability with three samples such as S1F, C2F, and R3F were used to determine the organoleptic properties. The questionnaires were prepared using 9 points hedonic scale

The points are stated below;

- 9 ---Like extremely
- 8 ---Like very much
- 7 ---Like moderately
- 6 ---Like slightly
- 5 ---Neither Like nor dislike
- 4 ---Dislike slightly
- 3 ---Dislike moderately
- 2 ---Dislike very much
- 1 ---Dislike extremely

Questionnaires were administered and scoring was done fortnightly.

Statistical analysis

Data were subjected to Statistical Analyses and the tool used is an analysis of Variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) version 22.0 software.

Physicochemical changes assessment

Trimethylamine

The procedure for determination of TMA concentration in fish was modified from those reported by Olanrewaju *et al.* (2017) [19]. 100mg fish was collected in a 2-mL tube, 1 mL of 10% trichloroacetic acid (at 4°C) was added and quickly homogenized with homogenizer (PRO SCIENTIFIC INC., USA). The liquid was transferred into a 2-mL tube, 1 mL of 10% trichloroacetic acid with vibration was added, and centrifugated at 3000 g for 3 min. The supernatant was then added to 0.2 mL of 10% formaldehyde solution, 2 mL anhydrous toluene, 0.6 mL 1:1 potassium carbonate (K₂CO₃) solution, shook up and down for 10 min; the liquid of the toluene phase was transferred to a plastic tube and 0.2 g sodium sulfate anhydrous was added and mixed properly. After mixing, 1 mL liquid was transferred to a new plastic tube and 1 mL of 0.02% picric acid was added, forming the yellow TMA-N-picric complex. 200 uL liquid was then transferred in a 96 well EIA/RIA plate (Corning Incorporated, USA) and measured photometrically at its maximum absorption wavelength of 410 nm in a spectrophotometer (TECAN infinite M200, Switzerland), with 10% trichloroacetic acid solution as blank control. A standard curve was produced from 14 TMA standard solutions (ranging in concentration from 0 to 50 µg/mL of TMA-N) to calculate the TMA concentration of each meat sample (R² =0.9984). The method was modified from Hultin (2004). 0.682 g of dry TMAH (C₃H₉N·HCl) was collected in a tube, 1 mL of HCl was added and dissolved with double distilled water up to 100 mL. 1.1905 mL was then taken from this solution and mixed with double distilled water up to 100 mL. That was the mother solution, and the concentration was 50 µg/mL TMA-N. The mother solution, was further diluted with double distilled water for 5, 10, 20, 30, 40 and 50 µg/mL respectively, which made the standard solution of TMA-N. The absorbance value obtained was 0.0698, 0.1089, 0.2394, 0.3342, 0.4484 and 0.5751 for each standard solution. The TMA concentration was then calculated from the TMA-N concentration (excel).

Dimethylamine

100mg fish was collected in a 2ml tube, 1 ml of 10% trichloroacetic acid (at 4°C) was added and quickly homogenized with homogenizer (PRO SCIENTIFIC INC., USA). The liquid was transferred into a 2-ml tube; 1 ml of 10% trichloroacetic acid with vibration was added, and centrifuged at 3000 g for 3 min. Aliquots of (0.1-4 ml) of extract was transferred into a tube. 0, 0.5, 1.0, 1.5, 2.0 and 2.5 ml of each working standard (DMA) solution was transferred into individual tubes to represent 0, 0.005, 0.01, 0.015, 0.020 and 0.025 mg amine nitrogen. Total volume was made to 4ml with distilled water for both extracts and standards. And the blank was 4.0 ml distilled water. 1ml 10% Formaldehyde, 10 ml toluene, and 3.0 ml 25% KOH was added to each. The temperature was ensued at 30°C, and the tubes was covered and mixed for 15 min. 7ml of upper toluene layer was removed using Pasteur pipette into

another drying tube containing approximately 0.3 to 0.4 g anhydrous Na₂SO₄ each. It was then Shaked gently (vortex mixer) until the solution was clear. 5 ml of picric acid working reagent was added into clean dry test tubes, then 5ml out of the 7ml obtained from above solution of toluene and anhydrous Na₂SO₄, was transferred into it and the absorbance was read at 410nm. The mother solution 0, 0.005, 0.01, 0.015, 0.020 and 0.025 mg amine nitrogen was respectively, which made the standard solution of DMA-N. The absorbance value obtained was 0.000, 0.101, 0.121, 0.201, 0.319 and 0.321 for each standard solution.

Thiobarbituric Acid

A stock solution of trichloroacetic acid (TCA), thiobarbituric acid (TBA) and Hydrochloric acid (HCl) containing 15g TCA, 0.375g TBA and 0.25N HCl was prepared. The solution was heated mildly to assist the dissolution of TBA. To 1ml of the tissue sample, 2ml of TCA-TBA-HCl mixture was added and heated in a boiling water bath for 15 minutes. It was cooled and then centrifuged at 3600rpm for 10 minutes. The absorbance of the supernatant was read at 532nm against a reagent blank which contained 3ml of the TCA-TBA-HCl solution. The malondialdehyde concentration was calculated as follows:

$$TBARS = \frac{\text{Absorbance} \times V \times 100}{A \times v \times Y}$$

Where

Absorbance is read at 532nm; V= total volume of the reaction mixture A= molar extinction coefficient which is 1.5x10⁵, Y= Weight of tissue; v= volume of sample used.

3. Results and Discussion

Table 1: Physicochemical changes of *Parachanna obscura* stored under refrigeration temperature at week two

Parameters	Raw	Smoked	Cooked
TMA	9.68 ^c	10.57 ^b	12.50 ^a
DMA	0.87 ^c	1.54 ^b	1.62 ^a
TBA	74.4 ^b	69.30 ^b	102 ^a

a,b,c, means with different superscript are statistically different

Table 2: Physicochemical changes of *Parachanna obscura* stored under refrigeration temperature at week four

Parameters	Raw	Smoked	Cooked
TMA	9.59 ^b	9.12 ^b	15.18 ^a
DMA	0.90 ^c	1.55 ^a	1.50 ^b
TBA	73.80 ^b	62.10 ^c	115.80 ^a

a,b,c, means with different superscript are statistically different

Table 3: Physicochemical changes of *Parachanna obscura* stored under refrigeration temperature at week six

Parameters	Raw	Smoked	Cooked
TMA	9.43 ^c	11.40 ^b	14.47 ^a
DMA	1.33 ^b	2.35 ^a	2.37 ^a
TBA	111.30 ^b	100.35 ^c	166.80 ^a

a,b,c, means with different superscript are statistically different

At week 2 the Trimethylamine (TMA) of *Parachanna obscura* were significantly (P<0.05) affected by processing method. Cooked *Parachanna obscura* had the significantly (P<0.05) highest value and raw *Parachanna obscura* had significantly list value.

Dimethylamine of *Parachanna obscura* stored under refrigeration temperature had similar trend with Trimethylamine as influenced by processing methods.

Thiobarbituric Acid of cooked *Parachanna obscura* was statistically higher than that of raw and smoked *Parachanna obscura* under refrigeration temperature which had significantly (P>0.05) similar mean values.

All parameters assessed at week 4 were significantly (P<0.05) affected by processing method. Trimethylamine of cooked *Parachanna obscura* were significantly higher than that of raw and smoked *P. obscura* and both have statistically (P>0.05) similar means values.

Dimethylamine of smoked *P. obscura* was significantly (P<0.05) higher than Cooked. Dimethylamine of raw was significantly (P<0.05) least across the three methods. Thiobarbituric Acid of cooked *P. obscura* was statistically (P<0.05) highest across the three while raw had significantly (P<0.05) least value.

At 6th weeks *Parachanna obscura* stored under refrigeration temperature with different processing method had significantly effect on the fish properties.

Trimethylamine of cooked samples was significantly (P<0.05) higher than other while the statistical (P<0.05) least value was obtained in rwa *P. obscura* fish. Dimethylamine of smoked and cooked *P. obscura* were statistically similar and are significantly higher than raw *P. obscura*.

Thiobarbituric Acid of cooked fish samples are significantly (P<0.05) highest, while raw fish had statistically (P<0.05) higher values than smoked *P. obscura*.

Table 4: Multiple Comparison of Parameters for week two

LSD						
Parameters	Parameters	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Texture	Taste	.6867*	.21151	.012	.1989	1.1744
	Colour	-.3600	.21151	.127	-.8477	.1277
	Flavour	.3533	.21151	.133	-.1344	.8411
	Overall acceptability	-.0867	.21151	.693	-.5744	.4011
Taste	Texture	-.6867*	.21151	.012	-1.1744	-.1989
	Colour	-1.0467*	.21151	.001	-1.5344	-.5589
	Flavour	-.3333	.21151	.154	-.8211	.1544
	Overall acceptability	-.7733*	.21151	.006	-1.2611	-.2856
Colour	Texture	.3600	.21151	.127	-.1277	.8477
	Taste	1.0467*	.21151	.001	.5589	1.5344
	Flavour	.7133*	.21151	.010	.2256	1.2011
	Overall acceptability	.2733	.21151	.232	-.2144	.7611
Flavour	Texture	-.3533	.21151	.133	-.8411	.1344
	Taste	.3333	.21151	.154	-.1544	.8211
	Colour	-.7133*	.21151	.010	-1.2011	-.2256
	Overall acceptability	-.4400	.21151	.071	-.9277	.0477
Overall acceptability	Texture	.0867	.21151	.693	-.4011	.5744
	Taste	.7733*	.21151	.006	.2856	1.2611
	Colour	-.2733	.21151	.232	-.7611	.2144
	Flavour	.4400	.21151	.071	-.0477	.9277

Based on observed means.

The error term is Mean Square (Error) = .067.

*. The mean difference is significant at the 0.05 level.

Table 5: Multiple Comparisons of Samples for Week two

Dependent Variable: Observation						
LSD						
(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
SIF	C2F	.1860	.16383	.289	-.1918	.5638
	R3F	-.2420	.16383	.178	-.6198	.1358
C2F	SIF	-.1860	.16383	.289	-.5638	.1918
	R3F	-.4280*	.16383	.031	-.8058	-.0502
R3F	SIF	.2420	.16383	.178	-.1358	.6198
	C2F	.4280*	.16383	.031	.0502	.8058

Based on observed means.

The error term is Mean Square (Error) = .067.

*. The mean difference is significant at the 0.05 level.

The table above shows that, the parameters (Texture, Taste, Colour, Flavour, and Overall Acceptability), as well as sample (SIF, C2F, and R3F), are highly significantly different since the (P<0.05).

Table 4 and 5 shows the multiple comparisons of the sample and the parameters. Based on the statistical analysis, there is a significant difference between the following parameters: Texture and Taste, Taste and Texture, Taste and Colour,

Taste and Overall acceptability, Colour and Taste, Colour and Flavour, Flavour and Colour, overall acceptability and Taste. Also, between the samples: C2F and R3F, R3F and C2F.

Table 5 in the multiple comparisons shows that there is a significant difference between C2F and Raw but C2F has an advantage over R3F since the difference value is negative.

Table 6: Multiple Comparisons of Parameters for Week four

Dependent Variable: Observation						
LSD						
(I) Parameters	(J) Parameters	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Texture	Taste	.1800	.32870	.599	-.5780	.9380
	Colour	-.6433	.32870	.086	-1.4013	.1146
	Flavour	.1333	.32870	.696	-.6246	.8913
	Overall acceptability	-.8033*	.32870	.040	-1.5613	-.0454
Taste	Texture	-.1800	.32870	.599	-.9380	.5780
	Colour	-.8233*	.32870	.037	-1.5813	-.0654
	Flavour	-.0467	.32870	.891	-.8046	.7113
	Overall acceptability	-.9833*	.32870	.017	-1.7413	-.2254
Colour	Texture	.6433	.32870	.086	-.1146	1.4013
	Taste	.8233*	.32870	.037	.0654	1.5813
	Flavour	.7767*	.32870	.046	.0187	1.5346
	Overall acceptability	-.1600	.32870	.639	-.9180	.5980
Flavour	Texture	-.1333	.32870	.696	-.8913	.6246
	Taste	.0467	.32870	.891	-.7113	.8046
	Colour	-.7767*	.32870	.046	-1.5346	-.0187
	Overall acceptability	-.9367*	.32870	.021	-1.6946	-.1787
Overall acceptability	Texture	.8033*	.32870	.040	.0454	1.5613
	Taste	.9833*	.32870	.017	.2254	1.7413
	Colour	.1600	.32870	.639	-.5980	.9180
	Flavour	.9367*	.32870	.021	.1787	1.6946

Based on observed means.

The error term is Mean Square (Error) = .162.

*. The mean difference is significant at the 0.05 level.

Table 7: Multiple Comparisons of Samples for Week four

LSD						
(I) Sample	(J) Sample	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
SIF	C2F	.6640*	.25461	.031	.0769	1.2511
	R3F	.0380	.25461	.885	-.5491	.6251
C2F	SIF	-.6640*	.25461	.031	-1.2511	-.0769
	R3F	-.6260*	.25461	.039	-1.2131	-.0389
R3F	SIF	-.0380	.25461	.885	-.6251	.5491
	C2F	.6260*	.25461	.039	.0389	1.2131

Based on observed means.

The error term is Mean Square (Error) = .162.

*. The mean difference is significant at the 0.05 level.

Table 6 and 7 shows the multiple comparisons of the sample and the parameters. Based on the statistical analysis, there is significant difference between the following parameters: Texture and Overall acceptability, Taste and Colour, Taste and overall acceptability, Colour and Taste, Colour and Flavour, Flavour and Colour, Flavour and Overall acceptability, overall acceptability and Texture, Overall

acceptability and Taste, Overall acceptability and Flavour. Also, between the samples: SIF and C2F, C2F and SIF, C2F and R3F, R3F and C2F.

Table 7 also shows that there is a significant difference between S1F and C2F but C2F also have an advantage over S1F, since the difference value is positive.

Table 8: Anova table for week six

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.381 ^a	6	.063	2.358	.130
Intercept	684.653	1	684.653	25440.752	.000
Parameters	.373	4	.093	3.462	.064
Sample	.008	2	.004	.149	.864
Error	.215	8	.027		
Total	685.249	15			
Corrected Total	.596	14			

a. R Squared = .639 (Adjusted R Squared = .368)

The above table shows that the parameters (Texture, Taste, Colour, Flavour, and Overall Acceptability), as well as a

sample (SIF, C2F, and R3F), are not significantly different since the (P>0.05).

Table 9: Multiple Comparisons of Parameters for Week eight

LSD						
Parameters	Parameters	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Texture	Taste	-.4000*	.11732	.009	-.6705	-.1295
	Colour	.0233	.11732	.847	-.2472	.2939
	Flavour	-.0633	.11732	.604	-.3339	.2072
	Overall Acceptability	-.3300*	.11732	.023	-.6005	-.0595
Taste	Texture	.4000*	.11732	.009	.1295	.6705
	Colour	.4233*	.11732	.007	.1528	.6939
	Flavour	.3367*	.11732	.021	.0661	.6072
	Overall Acceptability	.0700	.11732	.567	-.2005	.3405
Colour	Texture	-.0233	.11732	.847	-.2939	.2472
	Taste	-.4233*	.11732	.007	-.6939	-.1528
	Flavour	-.0867	.11732	.481	-.3572	.1839
	Overall acceptability	-.3533*	.11732	.017	-.6239	-.0828
Flavour	Texture	.0633	.11732	.604	-.2072	.3339
	Taste	-.3367*	.11732	.021	-.6072	-.0661
	Colour	.0867	.11732	.481	-.1839	.3572
	Overall Acceptability	-.2667	.11732	.053	-.5372	.0039
Overall acceptability	Texture	.3300*	.11732	.023	.0595	.6005
	Taste	-.0700	.11732	.567	-.3405	.2005
	Colour	.3533*	.11732	.017	.0828	.6239
	Flavour	.2667	.11732	.053	-.0039	.5372

Based on observed means.

The error term is Mean Square (Error) = .021.

*. The mean difference is significant at the 0.05 level.

Table 10: Multiple Comparisons of Samples for Week eight

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
SIF	C2F	.2780*	.09088	.016	.0684	.4876
	R3F	-.5360*	.09088	.000	-.7456	-.3264
C2F	SIF	-.2780*	.09088	.016	-.4876	-.0684
	R3F	-.8140*	.09088	.000	-1.0236	-.6044
R3F	SIF	.5360*	.09088	.000	.3264	.7456
	C2F	.8140*	.09088	.000	.6044	1.0236

Based on observed means.

The error term is Mean Square (Error) = .021.

*. The mean difference is significant at the 0.05 level.

Table 9 shows that, the parameters (Texture, Taste, Colour, Flavour, and Overall Acceptability), as well as sample (SIF, C2F, and R3F), are significantly different since the (P<

0.005).

Table 9 and 10 also shows the multiple comparisons of the sample and the parameters. Based on the statistical analysis,

there is a significant difference between the following parameters: Texture and Taste, Texture and Overall acceptability, Taste and Texture, Taste and Colour, Taste and Flavour, Colour and Overall acceptability, Flavour and Taste, Overall acceptability and Texture, Overall acceptability and Colour. Also, between the samples: S1F and C2F, S1F and R3F, and C2F and S1F, C2F and R3F, R3F and S1F, R3F and C2F.

Table 10 of Appendix 2 in the multiple comparisons results also shows that there is a significant difference in week 8 between S1F and C2F but C2F also have an advantage over S1F, since the different value is negative, C2F, S1F and R3F are significantly different but C2F also have advantages over them. Therefore, scientifically this research work has proved that C2F at refrigeration temperature as longer shelf life than S1F and R3F.

Table 11: Cultural and Biochemical characteristic of fish samples of steam cooked, smoked and fresh raw fish samples at week two

	Samples	Cooked (C2F)	Smoked (S1F)	Raw (R3F)
Morphological	Shape	Rod	-	-
	Size	small	-	-
	Edge	Entire	-	-
Microscopy	Elevation	Raised	-	-
	Surface	Rough	-	-
	Gram staining	+	-	-
Biochemical	Catalase	+	-	-
	Coagulase	+	-	-
	Hydrogen sulphide	-	-	-
	Citrate	+	-	-
	Urease	+	-	-
	Motility TEST	-	-	-
	Arrangement	Chain	-	-
	Sucrose	AG	-	-
	lactose	AG	-	-
	Glucose	AG	-	-
	maltose	-	-	-
	Probable isolate	<i>Bacillus subtilis</i>	-	-

KEYS: A = Acid production, + = Positive reaction -
AG = Acid and Gas production, - = Negative reaction.

Table 11: Cultural and Biochemical characteristic of fish samples steam cooked, smoked and fresh raw fish samples at week 4

	Shape	Round	Round	Rod	Rod
Morphological	Size	Small	Small	Small	Small
	Edge	Flat	Flat	Entire	Entire
	Surface	Smooth	Smooth	Raised	smooth
Microscopy	Gram StaininG	+	+	+	-
	Catalase	+	+	+	+
Biochemical	Coagulase	+	-	+	-
	Hydrogen sulphide	+	-	+	+
	Citrate	+	-	-	+
	Urease	+	-	+	-
	Motility Test	-	-	+	+
	Arrangement	Cluster	Cluster	Chain	Chain
	Sucrose	AG	AG	AG	AG
	lactose	A	A	AG	AG
	Glucose	AG	AG	AG	AG
	maltose	A	-	-	-
	Probable isolate	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Bacillus subtilis</i>	<i>Salmonella spp.</i>

KEYS: A = Acid production, + = Positive reaction -
AG = Acid and Gas production, - = Negative reaction.

Table 13: Bacteriological Assessment Result

Table 13.1: Total Bacteria count of steam cooked, smoked and fresh raw fish samples at week two

Sample	Suspected organism	Dilution factor	No. of colony	Population in cfu/ml.
Cooked (C2F)	<i>B. Subtilis</i>	10 ⁻⁴	2	2 X 10 ⁴
Smoked (S1F)	-	-	-	-
Raw (R3F)	-	-	-	-

The bacteria colony count in week 2 from the above table indicates that C2F (Steam cooked) has the highest microbial load, and the following organism which is *Bacillus subtilis*

which are mostly found in the soil and gastrointestinal tract of animal and human. While the S1F (smoked) and R3F has no bacteria.

Table 13.2: Total Bacteria count of steam cooked, smoked and fresh raw fish samples at week 4

Sample	Suspected Organism	Dilution Factor	No. of Colony	Population in Cfu/ML.
C2F	<i>S. aureus</i>	10 ⁻⁴	88	8.8 X 10 ⁴
S1F	<i>B.subtilis S. epidermis Salmonella spp</i>	10 ⁻⁴	31	3.1 X 10 ⁴
R3F	<i>Salmonella spp</i>	10 ⁻⁴	1	1.0 X 10 ⁴

The bacteria colony count in week 4 from the above table indicates that C2F (Steam cooked) has the highest microbial load, and the following organism which is *staphylococcus aureus*, followed by S1F (smoked) but also has the highest

number of organism identified such as; *B. subtilis*, *S. epidermis* and *Shigella*. R3F (fresh raw) has the list bacteria count and *Shigella* as the organism species.

Table 13.3: Total Bacteria count of steam cooked, smoked and fresh raw fish samples at week 6

Sample	Suspected organism	Dilution factor	No. of colony	Population in cfu/ml.
C2F	<i>S. aureus</i>	10 ⁻⁴	97	9.7 X 10 ⁴
S1F	<i>B. subtilis Salmonella spp.</i>	10 ⁻⁴	59	5.9 X 10 ⁴
R3F	<i>Salmonella spp.</i>	10 ⁻⁴	5	5.0 X 10 ⁴

The bacteria colony count in week 6 from the above table indicates that C2F (Steam cooked) also has the highest microbial load, and the following organism species of bacteria are identified *S. aureus*. Two organism's species are

identified in S1F (smoked fish) in week 6 which are *B. subtilis* and *Shigella spp.*, also in R3F (fresh fish) *Shigella spp.* are identified as the organism species of bacteria.

Table 13.4: Total Bacteria count of steam cooked, smoked and fresh raw fish samples at week 8

Sample	Suspected organism	Dilution factor	No. Of colony	Population in cfu/ml.
C2F	<i>S. aureus Shigella</i>	10 ⁻⁴	105	1.05 X 10 ⁴
S1F	<i>B.subtilis Shigella spp</i>	10 ⁻⁴	79	7.9 X 10 ⁴
R3F	<i>Shigella spp</i>	10 ⁻⁴	44	4.4 X 10 ⁴

The bacteria colony count in week 8 from the above table indicates that C2F (Steam cooked) also has the highest microbial load, and the organism species of bacteria identified also increased such as *S. aureus* and *Shigella spp.* S1F (smoked fish) has the following species of bacteria, *B. subtilis spp* and *Shigella spp*, also in R3F (fresh fish) *Shigella spp* is identified as the organism species of bacteria.

The results of this study also revealed that *Shigella* species, *Staphylococcus aureus*, *Bacillus subtilis* and *staphylococcus epidermis* were the most common bacteria found in fresh, smoked and steamed cooked fish stored under refrigeration temperature for the period of 8 weeks which also relate to the findings of Adeogun *et al.* (2008) [5]. According to the findings of Adewumi and Olaleye (2011) [6] which state that

the presence of *S. aureus* in the fish might be attributed to the contamination of the fish by man through handling and processing of the fish because its main habitat is human and animals which are mostly found in the nose, throat and skin of healthy individuals. *Bacillus subtilis* are mostly found in the soil and gastrointestinal tract of animal and human. *B. subtilis* spore can survive extreme temperature during cooking (Aubourg, *et al.*, 2007 Olagunju *et al.*, 2007) [7]. The pathogenic state of species of *staphylococcus aureus* is alarming. According Ayoade *et al.* (2003) he stated that *S. aureus* remain a major cause of food borne diseases. Enterotoxins produced by *S. aureus* are another serious cause of gastroenteritis after consumption of fish and *Shigella* species is the major cause of shigellosis (the earlier name was bacillary dysentery (Onyia, *et al.*,2013; Oyelese

et al., 2014) [20]. *Bacillus subtilis* as beneficial effect for growth promoter, for quality water control and immunity enhancement (Cakli *et al.*, 2016;). The *B. subtilis* are produced by C2F in week 2 only but S1F produced *B. subtilis* in week 4, 6, and 8 which give him more advantage over C2F (cooked) and R3F (raw) under the investigation of microbial load. It was observed that as the storage period progresses the bacterial level of C2F and SIF increases as well as the bacterial species.

4. Conclusion

The result of this present investigation concluded that *Parachanna obscura* can be stored for a period of 8 weeks at refrigeration temperature. The results obtained from the physicochemical changes, microbiological activities and sensory evaluation assessment which was examined fortnightly indicates that the longer the storage duration the more the quality of the fish are affected at refrigeration temperature. The microbial count during the storage was found to be within the required limit. However, *Parachanna obscura* preserved for a period of eight weeks are fit for human consumption having less microbial load.

5. Recommendation

Based on the result of this research work, smoked and frozen *Parachanna obscura* preserved in refrigeration for a period of 8 weeks without epileptic power supply are suitable for consumption. The total bacterial counts are found to be within the required limits according to international standard organization which is 5*10⁵ to 6*10⁵ in aerobic colony count.

6. Reference

1. Abassi DA, A Ogar. Proximate Analysis of snakehead fish, *Parachanna obscura* (Gunther 1863) of the cross river, Nigeria. *Journal of Fisheries and Aquatic Science*. Academic Journal Inc, 2012.
2. Abbas KA, Saleh AM, Mohamed A, O Lasekan. The relationship between water activity and fish spoilage during cold storage: A review. *Journal of Food and Agricultural Environment*, 2009; 7:86-90.
3. Adebawale BA, LN Dongo, CO Jayeola, SB Orisajo. Comparative quality assessment of fish (*Clarias gariepinus*) smoked with cocoa pod husk and three other different smoking materials. *Journal of Food Technology*. 2008; 6:5-8.
4. Adedeji OB, and RC Okocha C. Constraint to aquaculture development in Nigeria and way forward. *Veterinary Public Health and Preventive Medicine*, University of Ibadan, Nigeria, 2011.
5. Adeogun OA, Ajana AM, Ayinla OA, Yarhere M, Adeogun MO. Application of Logit Model in adoption decision: A study of hybrid *Clarias* in Lagos State, Nigeria. *American-Eurasian J. Agric. Environ. Sci.* 2008; 4(4):468-472.
6. Adewumi AA, Olaleye VF, Catfish culture in Nigeria: Progress, prospects, and problems. *Afr. J. Agric. Res.* 6(6):1281- 1285. Agriculture Organization, Rome, Italy. <http://www.fao.org/fishery/topic/12320/en>, 2011.
7. Aubourg SP, Quitral V, MA Larrain, A Rodriguez, Gomez J, Maier L, Vinagre J. Autolytic degradation and microbiological activity in farmed Coho salmon (*Oncorhynchus kisutch*) during chilled storage. *Food Chem.* 2007; 104:369-375.
8. Ayoade AA, Fagade SO, Adebisi AA. Dynamics of Limnological features of two man-made lakes in relation to fish production. *African Journal of Biotechnology*. 2006; 5(10):1013-1021.
9. Barbuzzi G, Grimaldi F, Del Nobile MA. The quality decay of fresh processed fish stored under refrigerated conditions. *J. Food Saf.* 2009; 29:271-286.
10. Berkel BM, Boogaard BV, Heijnen C. Preservation of Fish and Meat. Agromisa Foundation, Wageningen, the Netherlands, 2004, 78-80.
11. Bojanic K, Kozac-inski L, Filipovic I, Cvrtila Z, Zdolec N, Njari B. Quality of sea bass meat during storage on ice. *Meso.* 2009; 11:74-80.
12. Cakli S, Kilinc B, Cadun A, Dincer T, Tolasa S. Quality differences of whole ungutted sea bream (*Sparus aurata*), 2007.
13. Davies RM, Davies OA. Traditional and Improved Fish Processing Technologies in Values of Fish. (*Tropical Science*). 2009; 33:183-189.
14. Ekunwe PA, Emokaro CO. Technical efficiency of catfish farmers in Kaduna. *Nig. J. Appl. Sci. Res.* 2009; 5(7):802-805.
15. Engvang K, HH Nielsen, Proteolysis in fresh and cold-smoked salmon during cold storage: Effects of storage time and smoking process. *J. Food Biochem.* 10.1111/j.1745-4514.2001.tb00747.x. 2001; 25:379-395.
16. FAO. State of World Fisheries & Aquaculture 2006. Food and Agriculture Organization, Rome, 2006.
17. Obi SKC, Krakowiaka A. Theory and Practice of Food Microbiology (Unpublished manual), 1983.
18. Olagunju FI, Adesiyun IO, Ezekiel AA. The economic viability of catfish production in Oyo State, Nigeria. *J. Hum. Ecol.* 2007; 121(2):121-124.
19. Olanrewaju AN, Ajani EK, Kareem OK. Physico-Chemical Status of Eleyele Reservoir, Ibadan, Nigeria. *J. Aquac. Res. Development*, 8:9 Onyia, L.U., Michael, K.S., Manu, J.M. and Sabo, M. (2013). Comparison of Nutrient Values of Wild and Cultured *Heterobranchus bidorsalis* and *Clarias gariepinus*. *Nigerian Journal of Fisheries and Aquaculture* vol. 2017; 1(1):7-12.
20. Oyelese OA, Olutimehin IO, Iyanda DJ. Qualitative characteristics of hot smoked, cold smoked and oven-dried heterosis niloticus under cold storage temperature condition. *Journal of Biology, Agriculture and healthcare.* 2014; 4(10):2014.