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Qualitative Changes of Cold Smoked *Clarias gariepinus* at Ambient and Cold Storage Conditions

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Fish is a highly perishable product and major causes of spoilage are autolysis, bacterial decomposition and oxidative rancidity in fatty fishes. One of the cheapest form of fish preservation is smoking. Although deteriorative changes occur after smoking, the need to further freeze to extend the shelf life of the fish product is therefore expedient. Deteriorative changes and shelf-life of cold smoked *Clarias gariepinus* were studied at ambient temperature and under cold storage temperature of -250c for a period of twelve weeks. The organoleptic analysis, chemical analysis: peroxide value (P.V); free fatty acids (FFA) and total volatile base (TVB) and bacterial count/flora isolation were studied to determine the rate of spoilage. A total of sixty (60) live samples of Clarias gariepinus, average weight 200-300 g and cold smoked for 18 hours at a temperature of 300C were used for this experiment.
significant differences ($P<0.05$) were found in proximate composition values of fresh, cold smoked and fish stored for twelve weeks. The chemical assessment and bacteriological parameters also differed significantly. Higher values were recorded for all measured parameters at ambient temperature as compared to cold storage temperature of -250c. There was an increase in the crude protein (C.P) level due to loss of moisture during smoking. Fat content increased as a result

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of gradual oxidative rancidity of poly unsaturated fatty acids from zero weeks of initially smoked *Clarias gariepinus* to the 12th week. Total Volatile Base-Nitrogen values, Peroxide values (PV), Free Fatty Acid and Bacterial count values also increased.
Seven (7) species of bacteria were identified from the cold stored cold smoked fish while nine (9) species were identified at the ambient stored smoked fish, this showed that spoilage condition is faster in ambient storage rather than in the cold storage. There was significant difference ($P<0.05$) in the number of bacteria isolated under ambient condition (9 species) and cold storage (6 species) cold smoked *Clarias gariepinus*. Based on this, there is need for further cold storage of cold smoked *Clarias gariepinus* in order to increase its shelf-life.

Keywords: Cold storage; Ambient Storage; cold smoked; Clarias gariepinus.

1. INTRODUCTION

1.1 Importance of Fish

Fish is an important component of diet for people throughout the world and contributes about 45% of the animal protein consumed in Nigeria [1]. The demand for fish in Nigeria is on the increase as a result of the health benefits and increasing growing population and therefore any shortfall in fish availability will affect the animal protein intakes of people in tropical countries [2]. Fish is an extremely perishable food and a lot of physical and chemical changes occur during postharvest thereby leading to deterioration in the quality. Eyo [3] stated that, the main causes of spoilage are autolysis and bacterial decomposition. Oxidative rancidity is another factor contributing to spoilage in fatty fishes [4]. Spoilage comprises of bacterial and chemical changes that occur in the fish [5]. Of all the preventive measures to combat spoilage in fish, at domestic and local levels, smoking remains the cheapest and most preferred. As documented by Oyelese and Magawata [6], smoking improves aroma flavors and has preservative effects on fish. Eyo [2] stated that all preservative methods are geared towards making the conditions in the fish uncomfortable for the bacteria and reduction of chemical reactions in the fish.

Fish is available in most markets as either fresh, smoked, dried canned or frozen and as such the problem of scarcity is removed [2]. Fish freezing is a method of fish preservation in which the product is brought into contact with refrigerated air or refrigerated surfaces in a compartment. Freezing hinders the microbial stability of fish thereby extending its shelf-life. Fish preservation methods therefore facilitate long term storage, marketing and distribution of fish and fish products [2]. Thus, there is the need for the study of preservation techniques and determination of spoilage parameters to minimize losses, thereby increasing the quality of fish available for human consumption in many of the developing countries. This will help to mitigate dietary problems and health hazards caused by the intake of contaminated fish [7].

1.2 Fish Processing

Smoking involves the removal of moisture from fish to increase and improve the shelf-life. In more recent times, fish is readily preserved by freezing and the smoking of fish is generally done for the unique taste and flavor imparted by the smoking process.

Smoke drying of fresh fish is of utmost importance since fish is highly susceptible to deterioration immediately after harvest and to prevent economic losses [8]. The efficient preparation of fish is important when top quality, maximum yield and highest possible profits are to be achieved. Fish processing is done to enhance the acceptability of the product by the consumers and storage life of fish is also extended in the process.

Poor handling of fish leads to early deterioration and spoilage of fresh fish thereby causing rejection by the consumers and economic loss to the producer. Therefore, it is needed to discover the best processing method for the fish products, depending on the species, which can prolong the shelf-life of the final product especially if it has not been consumed immediately and also monitor the product over a period of time through organoleptic assessment and chemical assessment studies in order to determine the suitability for consumption over a period of time especially at cold storage.

2. MATERIALS AND METHODS

2.1 Sample Collection

Sixty (60) live samples of *Clarias gariepinus*, average weight 200-300 g, were purchased from

a fish farm and smoked whole with the gut content. The weight of the samples was taken before smoking and after smoking.

2.2 Experimental Procedure and Treatment

The fishes were cold smoked for a period of 18 hours at a temperature of 30°C. The samples were divided into two (2) batches and kept in two (2) different nylon bags. Batch 1 was cold stored at -25°C while batch 2 was stored under ambient temperature and kept on a shelf. The proximate analysis was carried out for fresh sample, the initial proximate analysis for the cold smoked sample and final proximate analysis at the end of twelve (12) weeks for ambient and cold stored samples.

The work outline was designed on the following bases:

- Cold smoked *Clarias gariepinus* at ambient temperature
- Cold smoked *Clarias gariepinus* at cold storage

2.3 Quality Assessment of Fish Samples

The following analyses were carried out on the processed fish samples:

i. Proximate analysis
ii. Chemical analysis
iii. Organoleptic analysis

Samples of the processed *Clarias gariepinus* were withdrawn bi-weekly to determine the extent to which changes have occurred and compare the storage duration to determine their shelf-life.

2.4 Statistical Analysis

Analysis of variance (ANOVA) was used to compare the means of proximate analysis of fresh and cold smoked *Clarias gariepinus* at ambient and cold storage; the mean of organoleptic assessment results of fish at ambient and cold storage; changes in peroxide values, free fatty acids, total volatile base nitrogen and microbial count of ambient and cold stored *Clarias gariepinus*. Microsoft Excel 2007 was used for graphical illustrations.

2.5 Proximate Analysis

Proximate analysis was done chemically according to the official methods of analysis described by the Association of official Analytical Chemist [9] on a dry matter basis to determine a general proximate composition of the fish before cold smoking and also bi-weekly.

2.6 Chemical Analysis

The processed samples of *Clarias gariepinus* were macerated and used for the following chemical analysis.

i. Peroxide value (P.V)
ii. Free fatty acid value (F.F.A)
iii. Total volatile Base-Nitrogen (T.V.B-N)

2.7 Sensory Evaluation

Samples were picked bi-weekly at random from the cold stored and ambient stored samples and subjected to a six man panel, trained on the organoleptic assessment of the processed samples. The parameters employed by the panels were as follows:

Characteristics of processed samples include:

1. Appearance : this include skin mucus, color and rigidity
2. Texture
3. Taste
4. Odor

Sterilized water was used to wash their mouth during tasting as well as their hands during the handling of samples to avoid any carryover of taste or any other contamination. Scores for each characteristic were on a numerical scale of 1-8.

SCORE PATTERN

Excellent – 1
Very good – 2
Good – 3
Fair – 4
Just fair – 5
Poor – 6
Very poor – 7
Bad – 8

2.8 Bacterial Assessment of Fish Sample

1.0 g of fish sample was suspended in 100 ml of sterile distilled water. The mixture was properly shaken and 1.0 ml of it was pipetted using a sterile pipette into another sterile universal bottle containing 9.0 ml of distilled water. This process was prepared for other sterile bottles such that at the end of the serial dilution, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} folds were obtained in respective bottles. Each was then plated using the spread

plating techniques on the plate count Agar (PCA). Fish sample (0.1 ml aliquots) were transferred using sterile pipette on the surface of the plate count Agar composing of 5.0 g yeast extract powder, 5.0 g tryptone, 1.0 g Dextrose, 15.0 g Agar volume/litre of distilled water and yeast extract peptone dextrose Agar (YEPA) which consists of 3.0 g yeast extract powder, 5.0 g peptone, 15.0 g Agar, 10.0 g dextrose, 0.05 g Streptomycin sulphate in 1litre of distilled water.

The above constituents of each medium were weighed and dispensed into a conical flask, plugged with cotton wool and covered with aluminum foil. These were boiled to give homogenous suspension and then sterilized in the autoclave for 15minutes at 121°C. The sterilized medium was left to cool and then poured into sterile petri dishes and allowed to solidify. After solidification, the petri dishes were inverted and incubated in that form in order to prevent contamination from water droppings. A sterile glass spreader was then used to spread the sample quickly. The plates were then incubated at 15°C for 24 hours and observed for the growth of microorganisms [10].

2.9 Microbial Count

The pure culture of each colony was obtained by using a sterile wire loop. The sterile wire loop was used to streak each separate colony onto a new solidify plate count agar (PCA) and yeast extract peptone dextrose agar (YEPA) plaster which were then incubated at 15°C for 24 hours. Stock culture of each organism were made and kept on agar slant at 4°C and they were sub-cultured from time to time [10].

3. RESULTS AND DISCUSSION

3.1 Proximate Analysis

As shown in Table 1, Moisture Content (63.05%) was highest in the fresh fish. This dropped to 23.66% after initial hot smoking. A significant ($P<0.05$) drop was also noticed at the end of 12 weeks of storage with a higher value of 19.36% in the cold stored cold smoked fish compared to 14.55% in the ambient stored fish. Also, the lowest Crude Protein (C.P) of 19.98% was recorded in the fresh fish. After the initial cold smoking, the C.P increased to 49.55%. At the end of 12 weeks a further rise to 62.58% was observed in the ambient stored fish much higher than 57.48% recorded in the cold smoked stored fish. The Crude Fibre (C.F) in the fish sample increased from 0.94% to 1.24% after initial cold smoking; a drop of C.F was recorded at the end of 12 weeks with ambient stored sample reducing to 0.003% and cold stored fish sample reducing also to 0.001%. Ash Content increased from 2.96% in the fresh fish sample to 3.85% in the initially smoked, to 7.56% in the ambient stored sample and 5.58% in the cold stored sample. Fat Content also increased initially from 12.88% (fresh fish) to 21.44% (initially smoked) and finally dropped to 11.35% (ambient stored) and 10.58% (cold stored) at the end of 12 weeks of storage for the cold smoked fish.

It can be observed from the above table that the crude protein (C.P) of the initial fresh fish (19.98%) was much lower than the initial cold smoked fish (49.55%), this occurred as a result of condensation of proteins resulting from water loss during smoking as reported by Ali et al. [11]; Mohamadu et al. [12] that moisture content loss explains the increase in crude protein in smoke-dried fish species. This is also evident by the C.P 62.58% and C.P 57.48% recorded respectively on final storage of the fish at ambient and cold storage respectively.

The increase in C.P recorded at the final stage of storage should be expected because it has been justified by the drastic drop in final moisture content as compared with the fresh fish; from 63.05% to 23.66% in the initial smoked fish and then to 14.55% in the final ambient fish and 19.36% in the cold smoked cold stored fish. The difference between the final high moisture value for cold stored fish (19.36%) and the final ambient stored fish (14.55%) could be as a result of water condensation in the refrigerator as discussed by Ali et al. [11]; Mohamadu et al. [12] and the results from this research are justified.

Salt content have a direct impact on water movement as discussed by Oyelese and Magawata [6]; and moisture content in *Clarias gariepinus,* being a fresh water fish could not be restricted, since there was no salt content in to restrict water absorption by the flesh of fish.

Suvanich et al. [13] discussed from findings in their work on the chemical and quality characteristics of channel catfish and stated that prolonged storage of fish increase the dry matter content per unit weight of fish after smoking of fresh fish. In line with their findings, it was observed that the Ash content had a higher value in the final storage at ambient temperature (7.56%) and cold storage (5.58%) conditions as

compared with the initial ash content of fresh fish (2.96%). The increase in the final percentages of Ash content under ambient condition and in the cold storage is justified by Suvanich et al. [13].

It was observed that the initial cold smoked fat content (21.44%) was higher than the fresh, although this value dropped to 11.35% in the final ambient storage and 10.58% in the final cold storage. This observation is in line with Horner [14] assertion that there might be high risk of rancidity during prolong storage conditions due to the fatty nature of the fish. The reduction of the fat content at the end of storage can be attributed to oxidation of poly-unsaturated fatty acids (PUFA) contained in the fish tissue to products such as peroxides, aldehydes and ketones and the free fatty acids [14]. The greater the degree of unsaturation (i.e. the greater the number of unsaturated fatty acids), the greater would be the tendency for fat oxidation (rancidity) even under cold storage for smoked fish products.

3.2 Microbial Count

Tables 2 and 3 shows the microbial count analysis for ambient stored and cold stored cold smoked *Clarias gariepinus.*

Also, a total of nine (9) bacteria species were represented under ambient storage condition for cold smoked *Clarias gariepinus* at the end of 12 weeks with a total count of 502 $cfug^{1}$. *Vibro cholera* had the highest load 87 $cfug^{1}$, followed by *Staphylococcus iscari* (80 $cfug^{-1}$) and third was *Staphylococcus luteus* (78 $cfug^{1}$).

Seven (7) bacteria species were represented under cold storage of cold smoked *Clarias gariepinus*. Also a lower $cfug^{1}$of 99 $cfug^{1}$ was recorded under cold storage compared to 502 $cfug^{1}$ recorded under ambient storage. However *Staphylococcus ischari* was highest with 30 $cfug^{1}$, followed by *Staphylococcus luteus* (29 $cfug^{1}$) and third was *Pasteureela haemolytica* with 20 $cfug^{1}$..

From Tables 3 and 4, the ambient microbial count (TVC) for the ambient stored *Clarias gariepinus* (1.46 $cfug^{-1}$/log10) was higher than that of cold stored *Clarias gariepinus* (1.27 $cfug^{-1}$/log10). Doe et al. [15] stated that for longer storage and better quality, fish may be frozen after smoking in the freezer for no longer than 2-3months. However, all the chemical and microbial results measured in this study were within the acceptable limits for human consumption.

Table 1. The initial and final proximate analysis of the fresh and cold smoked *Clarias gariepinus* at ambient and cold storage

Parameters	Fresh		Ambient storage		Cold storage
	Initial	Final	Initial	Final	
Moisture content (%)	63.05	23.66	14.55	23.66	19.36
Crude protein (%)	19.98	49.55	62.58	49.55	57.48
Crude fibre (%)	0.94	1.24	0.003	1.24	0.001
Ash content (%)	2.96	3.85	7.56	3.85	5.58
Fat content (%)	12.88	21.44	11.35	21.44	10.58
N.F.E (%)	0.19	0.26	3.96	0.26	7.00

Table 2. The microbial count analysis for ambient stored cold smoked *Clarias gariepinus*

Isolated microorganisms	Ambient storage weeks							
	0th wk	2nd wk	4th wk	6th wk	8th wk	10th wk	12th wk	Total
Pasteureela haemolytica (cfug-1)	Nil	4	-	-	-	-	-	4
Staphylococcus auricularis(cfug-1)	Nil	6	-	-	-	-	-	6
Staphylococcus lentus (cfug-1)	Nil	4	6	10	14	20	24	78
Staphylococcus iscari (cfug-1)	Nil	2	5	12	18	20	23	80
Escherichia coli. (cfug-1)	Nil	3	6	8	9	12	20	58
Vibrio cholerae (cfug-1)	Nil	4	9	12	17	21	24	87
Vibrio fluviales (cfug-1)	Nil	5	10	12	13	15	19	74
Aeromonas hydophila (cfug-1)	Nil	3	5	6	9	12	18	53
Citrovacter freundii (cfug-1)	Nil	4	7	9	12	15	15	62

3.3 Sensory Evaluation

Tables 4,5,6 and 7 shows the anova table of organoleptic assessment of cold stored cold smoked, cold smoked ambient stored, cold stored and ambient stored *Clarias gariepinus.*

The cold stored cold smoked fish organoleptic assessment showed better result with a value of 2.88 compared to the ambient stored fish sample with a value of 3.19. This assertion is further strengthened with the mean higher PV value of 1.37 meq/kg at ambient compared to 1.31 meq/kg under cold storage. Also, FFA (1.70%) at ambient is higher than 1.46% for cold stored cold smoked fish, TVB-3.90 MgN/100 g fish for ambient to a lower value of 3.31 MgN/100 g fish for the cold stored fish. Generally, significant ($P<0.05$) differences existed both at ambient and cold storage for all the parameters measured, with the ambient stored fish showing a faster rate of spoilage.

From above Table 4, significant differences ($P<0.05$) was observed in the appearance of fish cold stored in relation to the weeks of storage while taste, texture and odour showed no significant difference in relation to the weeks of cold storage. There was also no significant difference in the appearance, taste, texture and odor acceptance by the taste panel in relation to weeks of cold storage.

Table 3. The microbial count analysis for cold stored cold smoked *Clarias gariepinus*

Isolated micro organisms	Cold storage (weeks)							
	0th wk	2nd wk	4th wk	6th wk	8th wk	10th wk	12th wk	Total
Pasteureela haemolytica (cfug-1)	Nil	-	2	3	5	5	5	20
Staphylococcus lentus (cfug-1)	Nil	2	3	4	6	7	7	29
Staphylococcus iscari(cfug-1)	Nil	2	3	5	6	7	7	30
Escherichia coli. (cfug-1)	Nil	3	4	-	-	-	-	7
Vibrio cholerae (cfug-1)	Nil	2	-	-	-	-	-	2
Vibrio fluviales (cfug-1)	Nil	2	3	-	3	-	-	8
Aeromonas hydophila (cfug-1)	Nil	3	-	-	-	-	-	3

Table 4. ANOVA table for organoleptic assessment of cold stored fish

Parameters	SV	Df	SS	MS	F cal	P value
Appearance	A	4	46.07	11.52	8.86*	2.78
	B	6	6.99	1.17	0.9^{ns}	2.51
	Error	24	3.13	1.30		
	Total	34				
Taste	A	4	2.57	0.64	2.00^{ns}	2.78
	B	6	3.12	0.52	1.63^{ns}	2.51
	Error	24	7.64	0.32		
	Total	34				
Texture	A	4	2.99	0.75	1.88^{ns}	2.78
	B	6	3.58	0.60	1.50^{ns}	2.51
	Error	24	9.60	0.40		
	Total	34				
Odor	A	4	10.61	2.65	1.15^{ns}	2.78
	B	6	22.17	3.70	1.61^{ns}	2.51
	Error	24	55.13	2.30		
	Total	34				

α= 0.05 A= Weeks (Block); B= Taste panel (Treatment)

Table 5. ANOVA table for the organoleptic assessment of ambient stored fish

Parameters	SV	Df	SS	MS	Fcal	Pvalue
Appearance	A	4	4.80	1,20	3.15*	2.78
	B	6	3.42	0.57	1.50^{ns}	2.51
	Error	24	9.00	0.38		
	Total	34				
Taste	A	4	20.97	5.24	22.78*	2.78
	B	6	0.64	0.11	0.48^{ns}	2.51
	Error	24	5.57	0.23		
	Total	34				
Texture	A	4	26.84	6.71	17.66*	2.78
	B	6	2.69	0.45	1.18^{ns}	2.51
	Error	24	9.21	0.38		
	Total	34				
Odor	A	4	12.86	3.22	8.94^{ns}	2.78
	B	6	11.65	1.94	5.39^{ns}	2.51
	Error	24	8.58	0.36		
	Total	34				

α= 0.05 A= Weeks (Block); B= Taste panel (Treatment)

Table 6. ANOVA table for organoleptic assessment of ambient stored fish

Parameters	SV	Df	SS	MS	Fcal	Ftab
PV meq/kg)	A	1	0.02	0.02	0.33^{ns}	5.99
	B	6	3.12	0.52	8.67	4.28
	Error	6	0.36	0.06		
	Total	13				
FFA (%)	A	1	0.07	0.07	1.17^{ns}	5.99
	B	6	5.01	0.84	1.4*	4.28
	Error	6	0.36	0.06		
	Total	13				
TVB-N	A	1	0.60	0.60	1.09^{ns}	5.99
(mgN/100g)	B	6	26.78	4.46	3.91^{ns}	4.28
	Error	6	3.28	0.55		
	Total	13				
Microbial	A	1	0.27	0.27	13.5*	5.99
count(cfug^{-1})	B	6	3.70	0.62	3.1*	4.28
	Error	6	0.12	0.02		
	Total	13				

α= 0.05 A=Weeks (Block); B=Fish values (Treatment)

From the Anova Table 5, there were significant differences (P<0.05) in the appearance, taste, texture and odour of fish stored at ambient temperature in relation to the weeks of storage. Significant differences (P<0.05) also occurred in the odour acceptance by the taste panelist of ambient stored *Clarias gariepinus* while no significant differences (P>0.05) in appearance, taste and texture acceptance by the taste panelist in relation to the weeks of ambient storage.

From Table 6, significant differences (P<0.05) was observed in microbial count value (cfug^{-1}/log10) in relation to the weeks and significant differences (P<0.05) were also observed in PV, FFA, TVB and microbial count values among the fishes stored at ambient temperature.

Table 7. ANOVA table for organoleptic assessment of cold stored fish

Parameters	SV	Df	SS	MS	Fcal	Ftab
PV meq/kg)	A	1	0.04	0.04`	0.05^{ns}	5.99
	B	6	8.49	1.42	1.95^{ns}	4.28
	Error	6	4.4	0.73		
	Total	13				
FFA (%)	A	1	0	0	0^{ns}	5.99
	B	6	3.65	0.61	8.71	4.28
	Error	6	0.41	0.07		
	Total	13				
TVB-N	A	1	0.32	0.32	0.21^{ns}	5.99
(mgN/100g)	B	6	0.31	0.02	0.01^{ns}	4.28
	Error	6	9.12	1.52		
	Total	13				
Microbial	A	1	0.03	0.03	1.5^{ns}	5.99
count($cfug^{-1}$)	B	6	2.76	0.46	2.3^{ns}	4.28
	Error	6	0.11	0.02		
	Total	13				

α= 0.05 A=Weeks (Block); B=Fish values (Treatment)

Table 8. ANOVA results for chemical parameters under cold and ambient storage

Parameters	SV	Df	SS	MS	Fcal	Ftab
PV	A	1	0.02	0.020	0.081^{ns}	4.75
	B	5	0.11	0.022	0.089^{ns}	3.11
	AB	5	0.01	0.002	0.008^{ns}	3.11
	Error	12	2.96			
	Total	23	3.10			
FFA	A	1	0.35	0.350	25*	4.75
	B	5	0.08	0.016	1.143^{ns}	3.11
	AB	5	0.01	0.002	0.143^{ns}	3.11
	Error	12	0.17	0.014		
	Total	23	0.61			
TVB-N	A	1	2.07	2.070	1.198^{ns}	4.75
	B	5	0.59	0.118	0.068^{ns}	3.11
	AB	5	21.01	4.202	2.432^{ns}	3.11
	Error	12	20.73	1.728		
	Total	23	44.4			
Microbial count	A	1	0.07	0.070	0.579^{ns}	4.75
	B	5	0.19	0.038	0.314^{ns}	3.11
	AB	5	1.26	0.252	1.818^{ns}	3.11
	Error	12	1.45	0.121		
	Total	23	2.97			

α=0.05 A= Ambient and Cold storage B= Weeks

Table 9. Results of mean separations for proximate compositions and microbial counts for cold and ambient stored *C. gariepinus* in different weeks

Weeks/ Parameters	2^{nd}	4^{th}	6^{th}	8^{th}	10^{th}	12^{th}
PV	1.24 ± 0.01^{a}	1.28 ± 0.01^{a}	1.34 ± 0.05^{b}	1.37 ± 0.05^{b}	1.39 ± 0.06^{bc}	1.43 ± 0.05^{c}
FFA	1.46 ± 0.11^{a}	1.55 ± 0.17^{a}	1.59 ± 0.19^{a}	1.60 ± 0.20^{a}	1.62 ± 0.19^{a}	1.65 ± 0.17^{a}
TVBN	3.30 ± 0.11^{a}	3.57 ± 0.40^{a}	3.60 ± 0.42^{a}	3.67 ± 0.47^{a}	3.73 ± 0.53^{a}	3.76 ± 0.54^{a}
Microbial count	1.28 ± 0.08^{a}	1.33 ± 0.11^{a}	1.37 ± 0.16^{a}	1.39 ± 0.15^{a}	1.40 ± 0.16^{a}	1.42 ± 0.16^{a}

N.B: means with the same alphabets as superscripts are not significantly different from each other

Table 10. Results of analysis of variance for the proximate compositions and microbial counts in different weeks under cold storage

Parameters	Source	Df	SS	MS	F	P-value
PV	Week	5	0.0479	0.0096	5.25	0.034
	Error	6	0.0120	0.00183		
FFA	Week	5	0.0413	0.0083	0.27	0.912
	Error	6	0.1810	0.0302		
TVBN	Week	5	0.2810	0.0560	0.30	0.899
	Error	6	1.1420	0.1900		
Microbial count	Week	5	0.0262	0.0052	0.27	0.911
	Error	6	0.1147	0.0191		

α = 0.05

From Table 7, there was no significant difference (P>0.05) in FFA values, PV, TVB and microbial count values in relation to the weeks of cold storage. Significant differences (P<0.05) occurred in FFA values among stored fish.

3.4 Chemical Analysis

All the chemical parameters measured P.V, FFA and TVB increased under cold storage from 0-12weeks as shown on Table 1. Also, the P.V, FFA and TVB increased under ambient storage from 0-12weeks. Although, much higher values were recorded at ambient storage as shown on Table 8.

The result for the peroxide value (PV) reveals that the mean values for the 2nd and 4th week are not significantly different from each other for the two storage methods, but significantly differ from the 6th, 8th, 10th and the 12th week. Similarly, the mean values for the 6th, 8th and 10th week are not significantly different from one another. Also, the mean values for the 10th and 12th week are not significantly different from each other for the two storage methods. The results for the FFA, TVBN and microbial counts reveal that the mean values for all the weeks for the two storage methods are not significantly different from one another. Details of the results of the mean separations are shown in Table 9.

However, there is need for further cold storage of cold smoked *Clarias gariepinus* since nine (9) bacteria species isolated for the ambient (Table 2) were much higher and significantly different (P<0.05) from the six (6) bacteria species isolated under cold storage (Table 3) (Fungal growth were noticed as white moldy spec at the 10th week for ambient stored cold smoked fish while cold stored fish was intact with no fungal growth).

From the ANOVA results in Table 10, it was be observed from Factor A that there is a significant difference (P>0.05) in free fatty acid value and no significant differences (P<0.05) in the values of peroxide, microbial count and total volatile base of fish at cold and ambient storage. The interaction AB shows no significant variance component of fatty acids, TVB and Microbial counts. This indicated that the added variance components of weeks due to the interaction with fish are not significant. Factor B showed no significant difference in FFA, Peroxide values, TVB and Microbial count.

4. CONCLUSION AND RECOMMENDATION

Proper preservation methods are essential for prolonged shelf life and quality of fish products. From the study, it was observed that the cold stored cold smoked *Clarias gariepinus* was the best accepted in terms of consumer preference and higher quality in terms of lower values recorded for the chemical and bacteria assessment. It was also evident that the crude protein value had an inverse relationship with the moisture content and that recorded in the cold stored was higher than the ambient stored which can result from condensation of water in the refrigerator.

However, there is need for further preservation of cold stored *Clarias gariepinus* so as to maintain its quality evident, like salting which inhibits water movement into the fish and higher crude protein values can be recorded.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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