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Qualitative Characteristics of Hot Smoked, Cold Smoked and Ovenried Heterotis Niloticus under Cold Storage Temperature Condition

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Abstract

The quality characteristics of processed fresh Heterotis niloticus collected from Badagry Lagoon area of Lagos was investigated under different processing methods.

Hot smoked (at 80^0c at 12 hours per day for two days), Cold smoked (at 30^0c for 18 hours) and oven dried (at 60^0c for two days) with regular turning of the fish manually. Each of the processed samples (consisting of 20 samples in each treatment.) were separately kept in transparent nylon and put in a freezer for 12 week at -25^0c. Samples were taken fortnightly for various analysis. A total of 80 fresh Heterotis niloticus (descaled, degutted and washed with clean water) averaging 350 ± 0.88gm, total length 26.50 ± 0.64 cm) were collected for this study. Initial and final proximate analysis for the different processed fish samples were taken as well as chemical analysis (P.V, F.F.A and T.V.B-N), organoleptic assessment (appearance, taste, texture and odour) and microbial analysis (isolation, identification and count) were also examined at two weeks interval. The initial and final proximate analysis of cold smoked processed Heterotis niloticus had highest moisture content of 32.73% (initial), and 27.21% (final), protein (41.20% and 42.32%). Oven dried; moisture content (27.80%, 25.70%), protein (37.40%, 38.83%) while Hot smoked, also had a moisture content of (28.71%, 26.68%) crude protein (38.75% and 39.26%) respectively. Chemical parameters were significantly different $p<0.05$ for the three processing methods (cold smoked, hot smoked and oven dried as follows,. TVB-N (3.11, 2.54 and 2.60) mg N/100gm., FFA (5.21%, 4.68% and 4.85%) and PV mean values(4.31,3.75 and4.85) meq/kg respectively. The organoleptic assessment score was just satisfactory at eight week of storage, especially for odour, but Hot smoked samples was still good at 10^{th} week and oven dried samples was satisfactory. Microbial count (bacteria and fungi), the cold smoked product was significantly different at $p<0.05$ from the other two processing methods. Mean bacteria count was highest (70.14cfu/gm), followed by oven dried (2.14 cfu/gm) and least (2.00 cfu/gm) in hot smoked. Mean total microbial count was highest (575 cfu/gm) for cold smoked, 28 cfu/gm (oven dried) and the heat is 24 cfu/gm in hot smoked. The best processing method is hot smoking with the least microbial count 24 cfu/gm, T.V.B-N 2.54 mg N/100gm, F.F.A 4.68% and 3.75 meq/kg. spoilage increase with storage length with positive correlation (r) recorded for all the parameters in the 3 processing methods. Microbial count /isolation was not recorded for (0-8weeks) in hot smoked fish fish with Staphylococous aureus and Pseudomonas aeruginosa not represented throughout the 12weeks of study. In oven dried, bacteria and fungi were represented as from the 12^{th} weeks while only two fungi specie (Aspeigillus niger and Sacchromyces sp occurred only in the 10^{th} week. All the microbial species were represented through the 12weeks in the oven dried fish. Organoleptic assessment showed hot smoked with the best appearance, taste, texture and odour (with mean scores of (4.74, 5.02, 4.72, and4.79) followed by oven dried (4.62,4.71,4.70, and 4.64); while the worst processing method is cold smoked

(4.10, 3.95, 4.07 and 3.26) with just satisfactory (3.95 and 3.26) taste and odour at 12 week of cold storage at -25^{0}c
Keywords: Quality characteristics, Cold smoked, Oven dried.

Introduction

Fish preservation is a crucial aspect of biodiversity conservation of fisheries commerce in Nigeria, especially because of the high prevailing temperature which causes rapid deterioration of the fish caught in the River and lakes when marketed fresh, and by far the greater process done by smoking and drying (Osiyi 1979).
According to FAO (1976), the production of dried fish in the processing industry are classified as dried fish, dried salted fish, sun dried, and smoked dried fish. Methods of smoking include hot smoking (at temperature not exceeding 30^{0}c).

Quality is the degree of goodness of any product. In terms of fish, quality control definition involves all the fish attributes which the fish consumer and buyer considered important and necessary either consciously or unconsciously (Huss, 1988). Such attributes may include intrinsic meaning qualities that come naturally with the fish .

Intrinsic qualities include species, size, degree of contamination from handling, intrinsic quality can be influenced by extrinsic factors such as gaping of the fillets and other aesthetic considerations (Huss, 1988). The formation of lactic acid in the flesh of the fish after death effectually increases the degree of acidity.

The source of glycogen is the carbohydrates and this could be broken down into glucose, sugar phosphate pyruvic acid and slightly larger amount of lactic acid in most fish species.

The glycogen is important in fish rigor in that when fish go into rigor, the acidity in the flesh will rise according to the amount of glycogen present. It has been found that fish with a high post-mortern acidity have far better keeping qualities than those with a lower acidity and that their flavour is better as well (Bremmer,olles and Throng, 1978).

Water is a major component of all fish species, both in fresh and marine, water typically; the content is in the range of 67 to 80 percent of the fresh weight and may contain 90 percent in some deep water fish species. The high percentage of water in fish accounts for its perishability, most of the water in fresh fish is used either as a medium of transportation or for proliferation of bacteria.

Water also aid in the acceleration of lipase phase reaction and as such resulting into oxidative rancidity in fish. (Wory and Gill, 1987) did emphasis on the importance of water since most deterioration occurs in the fish muscle as a result of water medium for bacterial and microbial growth.

Traditionally prepared hot smoked products may be charged or burnt in extreme cases due to the uncontrolled nature of the process (Clucas, 1981).

There is no evidence that, the temperature typically reached during sun drying (up to 50^{0}c reported by mills) (1979) cause any appreciate loss of nutritional value of protein. However the exposure of wet fish to high temperature (75^{0}c-100^{0}c and above) may, most likely cause protein damage.

Thus in dried fish the protein may be less sensitive to heat damage, but more evidence is required before definite conclusions can be drawn as to the relationship between the temperature and time of treatment, moisture content of the fish and the degree of protein damage.

Therefore it would appear that, in the traditional curing techniques only hot smoking may lead to any significant protein damage.

It is therefore the objective of this study to investigate the quality characteristics of Heterotis niloticus under different processing methods (Hot smoked, cold smoked and oven dried) and determine the best method that will minimize the rate of loss in quality under cold storage temperature of-25^{0}c at the end of 12weeks.

2. Materials and Methods

2.1 Handling and preparation of fresh *Heterotis Niloticus*

Eighty fresh samples of Heterotis niloticus species with total body mean weight 350±0.88gm and total length 26.50±0.64cm were collected from Badagry Lagoon area of Lagos. They were transported in cellophane bags with ice and later put in a deep freezer to maintain the fresh quality before processing. They were descaled, degutted and washed with clean water, twenty samples were used for each processing methods (Hot smoked, cold smoked and oven dried). That is 3 treatments in all (i.e 60 fish samples). While the remaining 20 samples were kept for proximate analysis and other baseline (Chemical and microbial) studies such as peroxide value, free fatty acid, Total volatile basic nitrogen and microbial analysis.

2.2 Processing operations of the fish samples

The first batch of fish was hot smoked at 80^0c for 12 hours per day for two. The second batch was cold smoked at a temperature of 30^0c for 18 hours. While the third batch were arranged on another tray and put inside a gas oven for drying for two days at a temperature of 60^0c. Each treatment samples were separately kept in transparent nylon and put in a freezer for 12 weeks at-25^0c. Samples were taken fortnightly for various analysis.

2.3. Precaution and safety measures

At the point of processing, the following measures and precautions were strictly adhered to:

(i) The fresh Heterotis niloticus were carefully handled to prevent the fish from being bruised and contaminated.
(ii) The fish was descaled to allow heat penetration.
(iii) Clean and rust free knives were used.
(iv) The use of gloves, wearing of cap and laboratory coat and rubber solad shoes.
(v) Maintain best hygienic conditions.

2.4. Quality Assessments of the fish samples

The following four major analysis were carried out on the processed fish specie sample
(i) Proximate
(ii) Chemical analysis
(iii) Organoleptic assessment
(iv) Microbical (Bacteria and fungi) assessment.

Based on the above analysis, the processed samples of Heterotis niloticus were withdrawn on biweekly basis in order to determine how much changes have been affected and compare each level with the period or length of storage to determine their shelf-life.

2.5. Proximate Analysis

The proximate analysis was done on the processed samples of Heterotis niliticus and were analysed chemically according to the official methods of analysis described by the Association of official Analytical chemist (A.O.A.C, 2002). Initial and final proximate analysis of the Hot smoked, cold smoked and oven dried samples were determined
Parameters determined were crude protein content, crude fibre, moisture, ash, fat and Nitrogen free extract (NFE). Samples were withdrawn from the three major processing samples on the first day of analysis and subsequently fortnightly for further analysis.

2.5.1.Crude Protein content

Crude protein was determined by the Micro-Kjedahl method. This consists of three techniques of analysis Viz:- Digestion, Distilation and Titration'
The percentage Nitrogen in this analysis was calculated using the formular:

$$\%N = \frac{\text{Titre value x Normality of Hcl x Atomic mass of Nitrogen x Vol. of flask} \times 100}{\text{Weight of the sample digest (mg) x Vol. of digest (ml)}}$$

The crude protein content is determined by multiplying percentage nitrogen by constant factor of 6.25 i.e. percentage crude protein % = %N x 6.25

2.2.5 Crude Fibre Content

Samples were made after digestion of the food material in boiled dilute acid to hydrolyze the carbohydrate and protein followed by digestion in dilute alkali to effect saponification of the fat.

Samples were dried at 100^0C over night, Cooled in desiccators, and weighed (W_1.) samples were further put in furnace at 600^0C for 6hours, Cooled in desiccators and reweighed (W_2). The loss in weight during incineration represents the weight of crude fibre in the samples.

$$\% \text{ Fibre} = \frac{W_1 - W_2}{\text{Weight of sample}} \times \frac{100}{1}$$

2.5.3 Dry Matter and Moisture Content

The sample was weighed into a previously weighed crucible (W_0). The crucible plus sample taken was then transferred into oven set at 100^0C to dry to a constant weight for 24 hours overnight. At the end of 24hours the crucible plus sample was removed from the oven and transferred to desiccators, cooled for ten minutes and then weighed.

$$\text{Percentage dry matter } \% = \frac{W_2 - W_0}{W_1 - W_0} \times \frac{100}{1}$$

Where W_0 is the weight of empty crucible, $W_1$is the weight of crucible plus sample and W_2 is the weight of crucible plus oven dried sample.

$$\text{Percentage moisture } (\%) = \frac{W_2 - W_0}{W_1 - W_0} \times \frac{100}{1}$$

Or. % Moisture = 100 - % Dry matter

2.5.4 Ash Content

The sample was weighed into a porcelain crucible. This was transferred into the muffle furnace set at 550^0C and left for 4hours. The crucible and its content were cooled to about 100^0C in air at room temperature in desiccators and weighed. The percentage ash was calculated from the formula

$$\text{Ash Content } (\%) = \frac{\text{Weight of Ash}}{\text{Original Weight for Sample}} \times \frac{100}{1}$$

2.5.5 Crude Fat Or Ether Extracts

The percentage fat or oil is obtained by the formula below

$$\text{Fat content (\%)} = \frac{W_1 - W_0}{\text{Weight of sample taken}} \times \frac{100}{1}$$

WhereW_0 stand for initial weight of dry soxhlet flask, and W_1 stand for the final weight of oven dried flask plus oil or fat.

2.5.6 Nitrogen – Free Extract (NFE) Determination

The nitrogen free extract (NFE) calculation was made after the completion of analysis of the crude protein, crude fibre, moisture, ash and on other extract by adding the percentage values on dry matter basis and subtracting them from 100%

NFE = 100 - [(% crude protein) + (% crude fibre) + (% Moisture content) + (%

Ash Content) + (% Ether extract)]

2.6 Chemical Analysis

The chemical assessment of the processed samples of Heterotis niloticus were macerated and used for the following chemical analysis Viz (i) Peroxide Value (PV) (ii) Free Fatty Acid (FFA) (iii) Total Volatile Base – Nitrogen (T.V.B –N)

2.6.1 Peroxide Value (PV)

The fish sample was weighed into a clear dry boiling tube and 1.0gm of powdered potassium iodide and 20ml of solvent mixture (2 volume glacial acetic acid plus 1 volume of chloroform was added, the tube was placed on boiling water so that the sample boils within 30 seconds and allow to boil vigorously for not more than 30 seconds. The contents was quickly poured into a flask containing 20ml of potassium iodide solution (5%), the tube washed twice with 25ml of water and titrated with 0.002m sodium thiosulphate solution using starch as an indicator (1%). A blank was performed at the same time

$$\text{PV(meq/100gm} = \frac{\text{Titre value x Normality of acid used}}{\text{Weight of the sample}} \times \frac{100}{1}$$

2.6.2 Free Fatty Acids Determination

$$\text{Percentage free Fatty Acids} = \frac{\text{Titration value(ml)}}{\text{weight of sample used}} \times \frac{5.61}{1}$$

2.6.3 Total Volatile Base -Nitrogen

The fish sample was macerated with 100 ml of tap water and washed into the distillation flask with 200ml tap water, followed by addition of 2.0gm of magnesium oxide (Mg_2O) and few anti bumping agent added to the 500ml recurring flask followed by addition of two drops of screened methyl-red indicator.

The apparatus was connected up with receiving tube dipping below the boric acid solution. The distillation flask was then heated with gas flame from Bunsen burner to enable the mixture to boil for 10minutes (before distillation was carried out) for another 25minutes. The distillate was titrated with 0.1N sulphuric acid (H_2SO_4). The titre value was multiplied by 14 to obtain total volatile base nitrogen in mz N/100gm sample.

2.7 Organoleptic Assessment

A six man panel was set up and trained on the organoleptic assessment of the processed samples of Heterotis Niloticus. The samples were taken randomly from each processing method (Stored fish), put in a sterilized- dried white plate on a table for assessment.

2.7.1 Characteristics Of Processed Heterotis Niloticus Assessed

The panel members were instructed to consider and take cognizance of the following characteristics Viz: 1) Appearance (2) Taste (3) Texture (4) Odour; to be used for the assessment on the three levels; of the processed fish samples. Panel members were sure sterilized water to wash their mouth and hands to avoid any carry over taste during assessment.

Evaluation score key: (1) Excellent-7 (2) Very Good-6 (3) Good -5 (4) Satisfactory-4 (5) Just satisfactory-3 (6) Fair-2 (7) Poor-1 (8) Unacceptable-0

3.8 Bacterial Assessment Of The Fish Sample.

1.0gm of fish sample was suspended in 100ml of sterile distilled water. The mixture was properly shaken and 1.0ml of this was pipetted using a sterile pipette into another sterile universal bottle containing 9.0ml of distilled water. This process was prepared for the other sterile bottle so that at the end of the serial dilution 10^{-1}, 10^{-2}, 10^{-3} 10^{-4}, and 10^{-5} folds were obtained in respective bottle. Each was then plated by spreading plating techniques on the plated count Agar (PCA). Fish sample (0.1ml aliquots) were transferred using sterile pipette on the surface of the plate count Agar composing of 5.0gm yeast extract, 5.0gm tryptone,1.0gm Dextrose,15.0gm Agar vol/liter of distilled water and yeast Extract peptone dextrose Agar (YEPA) consisting of 3.0gm yeast extract powder, 5.0gm peptone, 15.0gm Agar, 10.0gm dextrose, 0.05gm Streptomycin sulphate in/ litre of distilled water.

The above constituent of each medium were weighed and dispensed into a conical flask, plugged with cotton wool and covered with aluminum foil. These were boiled to sure homogenous suspension and then sterilized in the autoclave for 15 minutes at 121^0c. 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 to prevent contamination form water droppings. A sterile glass spreader was then used to spread the sample quickly. The plate were then incubated at 15^0C for 24 hours and observed for the growth of micro-organism (Van Demark, 1972).

3.8.1 Microbial Count.

The pure culture of each colony was obtained by using a sterile wire loop. A sterile wire loop was used to streak each separated colony into a new solidify plate count agar (PCA) and yeast extract peptone.

Dextrose agar (YEPA) plaster was incubated at 15^oc for 24 hours. Stock culture of each organism were made and kept on agar plate at 4^oc and there were subcultured from time to time (van Demark, 1972).

3.9 Statistical analysis

Analysis of variance (ANOVA) in completely randomized design was performed on the data obtained using SPSS (2006). Significant means were compared at 5% probability level using Duncan's multiple range test (DMRT).

Result

Table 1- Proximate analysis of fresh and final hotsmoked, cold smoked and oven dried *Heterotis niloticus* under cold storage.

Parameter	Fresh	Hot Smoked		Cold Smoked		Ovendried	
		Initial	Final	Initial	Final	Initial	Final
Moisture Content(%)	34.78	28.71	26.68	32.73	27.21	27.80	25.70
Crude Protein(%)	40.01	38.75	39.26	41.20	42.32	37.40	38.83
Crude Fibre(%)	2.33	2.28	4.12	1.54	2.23	2.16	3.13
Ash Content(%)	11.20	14.05	14.75	8.58	13.97	15.37	17.41
Fat Content(%)	10.12	14.28	12.60	13.23	12.61	13.60	13.00
	1.56	1.93	2.59	2.72	1.66	3.67	1.93

Table 1 – shows the cold smoked Heterotis niloticus initially had the highest moisture content followed by hot smoked, while the least is ovendried as follows 32.73, 28.71 and 27.30. Crude protein value was highest (41.20%) in the initially smoked fish, followed by 38.75% in hot smoked and the least in ovendried (37.40%). Protein and fat were generally reduced in the final at the end of 12 weeks in all case. Also ash and fibre content increased, with the highest crude fibre of 4.12% (hot smoked) and 3.13 (ovendried) recorded respectively.

In the fresh Heterotis niloticus, moisture content and crude protein values of 34.78%_and 40.0% recorded were much higher than in all the values recorded for the three processing methods except for initial and final crude protein of the cold smoked product.

The crude protein values of the initial and final smoked product in all the three processing method were very close possibly because of the cold storage at 25^0C. Thus this is also responsible for the unpronounced condensation of protein in all cases.

Table2- Mean organoleptic assessment of hot smoked, cold smoked and oven dried *Heterotis niloticus* at the end of 12 weeks of cold storage at – 25^{0}c.

Parameters	Hot Smoked	Cold Smoked	Oven dried
Appearance	4.74 ± 0.9163	4% ± 0.7546	4.62 ± 0.5268
Taste	5.02 ± 0.5755	3.95 ± 0.7303	4. 71 ± 0.5639
Texture	4.72 ± 0.4592	4.07 ± 0.7303	4.70 ± 0.5026
Odour	4.79 ± 0.5405	3.26 ± 0.9035	4.64 ± 0.5165

In Table 2, organoleptic assessment showed hot smoked fish with the best appearance, taste, texture and odour with mean scores of (4.74, 5.02, 4.72 and 4.79) respectively, followed by ovendried (4.62, 4.71, 4.70 and 4. 64) while the worst processing method is cold – smoked (4.10, 3.95, 4.07 and 3.26) with just satisfactory (3.95 and 3.3.95 and 3.26) taste and odour at 12 weeks of cold storage at - 25^{0}C. Significant differences at ($P < 0.05$) for the quality assessed in the three processed fish samples, that is significantly, there is a change with the time of storage in the quality of organoleptic assessment of the processed fish samples.

The rate of deterioration was higher in the cold smoked processed Heterotis niloticus fish sample, followed by ovendried, while hot smoked processed fish was the best.

Table 3 – Mean chemical analysis values of hot smoked, cold smoked and ovendried heterotis niloticus at the end of 12 weeks cold storage at – 25^{0}c

Parameters	Hot Smoked	Cold Smoke	Ovendried
(1) Peroxide Value			
(Meq/Kg) (PV)	3.75	4.31	4.14
(2) Free Fatty Acid			
(%) (FFA)	4.68	5.21	4.85
(3) Total Volatile			
Base – Nitrogen	2.54	3.11	2.60
mg N/100gm$_{(TVBN)}$			

Chemical parameters (in Table 3) shows the cold smoked product is the worst preserved at the end of 12 weeks with (PV – 431meg/kg, FFA – 5.2%, TVB-N 3.11mgN/100gm, this is followed by ovendried with (PV – 14.14mg/kg, FFA -4.85%, TVB – N2.60mgN/100gm. While the best processing method fare the lowest values (PV – 3.75meg.kg, FFA – 4.68%, TVB – N2.54mgN/100gm) for the chemical parameters.

Table 4 - The microbial count analysis of hot smoked heterotis niloticus under cold storage (at - 25^0c) for 12 weeks.

Isolated Micro Organism	Type	HOT SMOKED							
X10^4 cfu/gm		0th wk	2nd wk	4th wk	6th wk	8th wk	10th wk	12th wk	TOTAL
(1) Staphylococcus aureus cfu/gm	BACTERIA	0	0	0	0	0	0	0	0
(2) Bacillus cereus cfu/gm		0	0	0	0	0	0	2	2
(3) Bacillus furinus cfu/gm		0	0	0	0	0	0	3	3
(4) Proteus vulfaricus cfu/gm		0	0	0	0	0	2	2	4
(5) Pseudomonas aureginosa cfu/gm		0	0	0	0	0	0	0	0
(6) Bacillus maceraus cfu/gm		0	0	0	0	0	1	4	5
(7) Fusaruim Oxysporum cfu/gm	FUNGI	0	0	0	0	0	0	0	0
(8) Aspergillus niger of cfu/gm		0	0	0	0	0	0	2	2
(9) Aspergillus terraus cfu/gm		0	0	0	0	0	0	3	3
(10) Sacchomyces Sp. cfu/gm		0	0	0	0	0	0	2	2
		0	0	0	0	0	0	3	3
		0	0	0	0	0	0	0	0

TOTAL		0	0	0	0	0	3	21	24

Table 5 - The microbial count analysis of cold smoked heterotis niloticus under cold storage (at - 250c) for 12 weeks.

Isolated Micro Organism	**Type**	**COLD SMOKED**							
X10^4 cfu/gm		**0th wk**	**2nd wk**	**4th wk**	**6th wk**	**8th wk**	**10th wk**	**12th wk**	**TOTAL**
(1) Staphylococcus aureus cfu/gm	BACTERIA	0	4	18	19	23	26	33	123
(2) Bacillus cereus cfu/gm		0	2	20	21	22	22	22	110
(3) Bacillus furius cfu/gm		0	1	12	14	16	17	21	81
(4) Proteus vulgaricus cfu/gm		2	3	9	10	12	14	18	68
(5) Pseudomonas aureginosa cfu/gm		2	2	10	11	12	13	15	65
(6) Bacillus maceraus cfu/gm		0	0	4	7	9	11	13	44
(7) Fusaruim oxysporum cfu/gm	FUNGI	0	0	1	1	3	5	7	17
(8) Aspergillus niger cfu/gm		0	0	0	2	2	4	6	14
(9) Aspergillus		0	0	0	0	0	2	3	5

terraus cfu/gm (10) Sacchomyces Sp. cfu/gm		0	0	5	7	10	12	14	48
TOTAL		4	12	79	92	109	126	153	575

Table 6 - The microbial count analysis of oven dried *Heterotis niloticus* under cold storage (at - 25^0c) for 12 weeks.

Isolated Micro Organism	**Type**	**OVEN DRIED**							
X10^4 of u/8m		**0th wk**	**2nd wk**	**4th wk**	**6th wk**	**8th wk**	**10th wk**	**12th wk**	**TOTAL**
(1) Staphylococcus aureus cfu/gm	BACTERIA	0	0	0	0	0	0	1	1
(2) Bacillus cereus cfu/gm		0	0	0	0	0	0	2	2
(3) Bacillus furinus of u/8m		0	0	0	0	0	0	3	3
(4) Proteus vulgaricus cfu/gm		0	0	0	0	0	0	2	2
(5) Pseudomonas aureginosa cfu/gm		0	0	0	0	0	0	3	3
(6) Bacillus macereus cfu/gm		0	0	0	0	0	0	4	4
(7) Fusaruim oxysporum cfu/gm	FUNGI	0	0	0	0	0	0	2	2
(8) Aspergillus cfu/gm		0	0	0	0	0	2	3	5

niger cfu/gm		0	0	0	0	0	0	2	2
(9) Aspergillus terraus cfu/gm		0	0	0	0	0	2	2	4
(10) Sacchomyces Sp. cfu/gm									
TOTAL		0	0	0	0	0	4	24	28

Table 7- The bacterial count (x104 cfu/gm) for all the processed *Heterotis niloticus* under cold storage (-25^0 c) for 12 weeks.

Treatment	0th wk	2nd wk	4th wk	6th wk	8th wk	10th wk	12th wk	MEAN
Hot smoked	0	0	0	0	0	3	11	2.00
Cold smoked	4	12	73	82	94	103	123	70.14
Oven Dried	0	0	0	0	0	0	15	2.14

Table 4, 5, 6 and 7 shows there is significant difference in the microbial count at p< 0.05 for all the three processed Heterotis niloticus. There is no significant difference P> 0.05 between Hot smoked (28 cfu/ gm– total microbial count) and over dried (24 cfu/ 8m – total microbial count) compared to 5.75 cfu/gm total microbial count for cold smoked Heterotis niloticus. Also the mean bacteria count followed the same order with cold smoked having the highest (70.14 cfu/ gm), followed by 2.14 in over dried and 2.00 in Hot smoked, showing cold smoked is significantly P< 0.05 different from the other 2 processing methods. There was no microbial count recorded from the 0th -8th week in oven dried and hot smoked Heterotis niloticus.

In cold smoked processed Heterotis niloticus samples, bacteria and fungi were detected all through the period of experiment except Staphylococcus aureus Bacillus ceraus, Bacillus furinus, Bacillus maceraus (all bacteria species) Fusarum oxysporum, Sacchomyces sp (fungi specie) in week 0 and 2. Four fungi species, including Aspergillums nipper and Aspergillums terraus were not detect in the 0th and 2nd week.

Table 8- Pearsons correlation coefficient(r) for p.v, ffa, tvb- n and microbial count of the processed heterotis niloticus under cold storage at(-25^{0}c) with length of storage.

CONSTITUENT	CORRELATION COEFFICIENT(R)		
	Hot Smoked	Cold Smoked	Oven Dried
Peroxide Value (meq/kg)	0.0309	0.9329	0.0279
Free fatty Acid (%)	0.9707	0.9829	0.9672
Total Volatile Base Nitrogen (MgN/ 100gm)	0.9360	0.9756	0.9691
Microbial count (cfu/ gm)	0.6800	0.9717	0.6901

Table 8 shows that there is a strong positive linear correlation relationship between the storage period and the chemical indices microbial count. It also means that as the length of storage increase, the chemical indices as well as microbial count increases.

Therefore, it can be inferred that bacteria is the precursor of these indices of spoilage, alteration in the free fatty acid, peroxide value and total volatile base nitrogen content.

Discussion

The processing method employed had great effect on the storage and shelf life final product of the processed Heterotis Niloticus fish samples, as the length of storage increases, the chemical indices as well as bacterial count increases.

There was a gradual increases in the concentration of Total Volatile Base- Nitrogen (TVB-N), peroxide values (PV) and free fatly Acid (FFA) and this support the work of Pearson (1982). He also reported that fish can be considered as fresh if the amount of TVB-N and PV is less than 20mg N/100gm fish and 40meq/kg of fish respectively and these fall within the ranges as it is presented in Tables 3. Also positive corrections (r) were recorded for all the chemical indices and bacterial count (Table 7) as length of storage increases for all the three processing methods.

Organoleptic assessment shows that the limit of acceptability which was just satisfactory for cold smoked processed Heterotis niloticus was the sixth week and the 10th week for hot smoked and oven dried samples. The tenth week also corresponded to the time when microbial growth was noticed in the hot smoked and oven dried fish samples, although they were still at satisfactory levels.

Further cold storage of the smoked products reduced the rate of deteriorative and maintained a reasonable level of quality of the hot smoked and oven dried Heterotis niloticus samples.

Hot –smoking is the best processing method with lowest microbial count 24x10^4 cfu/gm followed by cold smoking (28x10^4 cfu/gm) and lastly cold smoked (575x10^{4}cfu/gm)

Conlusion

Hot smoked Heterotis niloticus is the best with the least mean bacteria count of $2.0x10^4$ cfu/gm and least. TVB-N, and FFA values at the end of 12 weeks storage at (-25^0c). Also further cold storage enhances flavour, preserves texture, and reduces the rate of deterioration of smoked products.

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