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Quality Assessment of Cold Smoked Hot Smoked and Oven Dried *Tilapia nilotic*a Under Cold Storage Temperature Conditions

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Abstract: This study was designed to determine the best processing method (cold smoking, hot smoking and oven drying) that will minimize the loss of quality of *Tilapia nilotica* under cold storage temperature of -25°C. Fresh *Tilapia nilotica* were immediately chilled to keep their fresh quality intact. The chilled samples (of average size 185 gm) were subjected to different processing methods (cold smoking, hot smoking and oven drying) after which they were kept in 3 different sterilized containers and put in a freezer for 3 months at -25°C, while samples were taken bi-weekly for chemical analysis. A baseline data of all measured parameters were taken at the beginning (0 day) of the experiment. Also each of the 3 processed samples was subjected to organoleptic assessment, chemical analysis (P.V., F.F.A. T.V.B.-N) and microbial analysis (Isolation, Identification and count). Initial and final proximate analyses were carried out for each of the processed samples. The microbial analysis was carried out on a monthly basis after the initial baseline data was recorded for the fresh fish sample. Proximate composition for both the fresh fish, initial and final fish samples showed no significant variation ($p>0.05$). Significant variations ($p<0.05$) were obtained for all measured chemical parameters (P.V., F.F.A. T.V.B.-N microbial count and organoleptic assessment between the three processing methods. The best processing method was hot smoking with zero (0) microbial count, P.V. 3.9 meq kg^{-1}, F.F.A 7.0% and T.V.B.-N 3.02 mg N100 gm^{-1} fish. Hot smoked products had no microbial count under cold storage conditions of-25°C through out the experimental period. This was followed by oven drying, which showed least bacterial count of 2 cf ug^{-1} up till the 4th week of cold storage. The notable bacterial detected was *Staphylococcus aureus* (1 cf ug^{-1}) and *Bacillus subtilis* (1cf ug^{-1}). Lastly the poorest processing method is cold smoking with the highest bacteria count of 150 cf ug^{-1} where bacterial species were detected in the first 8 weeks of the three months storage period . However, the last 4 weeks of the experiment showed no traces of bacteria for the 3 processing methods. The prominent bacterial species in the cold smoked samples are *Staphylococcus aureus* (20cf ug^{-1}) and *Bacillus subtilis* (40 cf ug^{-1}) *Psendomonus aureginosa* (30 cf ug^{-1}) and *Micrococus acidiophilus* (35 cf ug^{-1}).

Key words: *Tilapia nilotica*, processing methods, bacteria count/identification, cold storage (-25°C), quality assessment

INTRODUCTION

Clucas[1] stated that fish is an extremely perishable foodstuff and as soon as the fish dies, scores of complicated changes arise, mainly by enzymic and bacteria action. Emokpae[2] also, reported that when a fish dies a number of physical and chemical changes leading to spoilage takes place in its body.

The three main stages that affect the quality of fish postmortem are the release of mucus, autolysis and bacterial decomposition. Emokpae[2] also noted that the release of mucus is a result of a particular reaction of the dying fish to an unfavourable surrounding. The slime secreted consists of gluco-protein mucin, which is an effective substrate for bacterial growth. As a result it soon putrefies and gives off offensive odours.

Pusztaiv[3] reported that animal tissue under stress is easily attacked by micro-organism. Nair[4] showed that putrefactory odours are mainly produced by bacterial metabolites invading amines indole, hydrogen sulphide and aldehyde. Excessive growth of bacteria leads to the repulsive appearance of spoilt fish Nour[4].

Fresh fish microbial count: When fish are landed, micro-organisms are found on all the outer parts of the fish (skin and gills) as well as in the intestines of live and newly caught fish. The total number of microorganism vary enormously and Liston[5] states a normal range of 10^2-10^7 cf ug^{-1} (Colony forming units)/cm^2 on the skin surface. In a study conducted by Shewan the gills and the intestine both contained microbial counts between 10^3 and 10^9 cf ug^{-1}. When these organisms are allowed to

grow and multiply, it will cause a rapid loss of the fresh quality and eventually lead to the spoilage of the dead fish. In practice, viable counts on marine fish, using salt water media, are only slightly higher than counts on fresh water media, provided there is salt (0.5%)[6]. In general, the gram-negative flora have faster growth rate at lower temperature than the gram-positive flora, which is particularly true below -5°C Hobbs[6]. Fish become contaminated with microorganisms at sea, in rivers or lakes and on landing Waterman[7].

Trimethylamine in fresh fish: The generally accepted method for the assessment of quality in seafood products is sensory analysis[8] Advantages of sensory analysis are its simplicity and rapidity. However, there are also several disadvantages such as the extensive training required for the panel members to avoid subjectivity[9]. These drawbacks have catalyzed research into the development of objective methods using chemical indicators to reinforce the conclusions reached by sensory analysis[10].

In a joint project, different European countries have been trying to develop Flow Injection/Gas Diffusion methods (FIGD) for trimethylamine and total volatile basic nitrogen in fish[8]. Flow injection analysis seems to be a good alternative tools for optimizing routine analyses, mainly because it offers the advantages of simplicity, low cost and rapidity Leon[11]. It has been described as an objective method with the same advantage as sensory analysis Ryder[12].

Trimethylamine oxide in fresh fish: Trimethylamine Oxide (TMAO) is a natural and nontoxic substance, generally involved in the osmoregulatory function of marine species of fish and shell fish[13]. The TMAO content in fresh fish varies within species and usually decreases after death. Trimethylamine oxide is mainly reduced by bacteria enzymes to trimethylamine, which is largely responsible for the characteristic off-odour of dead marine fish[14]. During fish storage, the increase in trimethylamine has been widely correlated with a decreasein TMAO concentration Rodriques[15].

MATERIALS AND METHODS

The freshly caught *Tilapia nilotica* from the dam of the Department of Fisheries, Oyo State Ministry of Agriculture Agodi, Ibadan were randomly packed into colourless cellophone bags at an amount of 1 kg per bag and kept at -25°C to prevent it from spoilage and to maintain the quality of fish for some hrs. The fish were then packed in 10 bags with ice and put in sterilized coolers for onward transportation to the processing unit only fish of relatively big size were selected for this study. A total number of 50 fish of average size 185 gm, of the fish that were big enough, were used for the present study. A number of 48 fish out of the 50 fish sorted for the experiment were divided into 3 batches with 16 fish per batch for the processing. The remaining 2 out of the 50 samples were put in an ice box for the proximate analysis and other baseline studies Peroxide Value (P.V); Free Fatty Acid (F.F.A); Total volatile Base- Nitrogen (TVB-N); microbial analysis) in the laboratory.

Processing operation: The first batch of fish was set aside for cold smoking, the second batch for hot smoking and the third batch for oven drying. The first and second batches of *Tilapia nilotica* were arranged on two different smoking trays and put on the fire made of firewood. The 3rd batch of the fish were arranged on another tray and put inside a gas oven for drying.

The 3rd batch of the fish was oven dried for 3 h at a temperature of 60°C for 2 days with regular manual turning of the fish. The 1st batch of the fish set aside for cold smoking was smoked on the fire for 1 hur during two consecutive days and the 2nd batch was smoked for 3 h for 2 days at the same temperature. After smoking and drying, each batch was put in sterilized containers marked A,B and C covered and stored at -25°C.

Quality assessment of the samples: Four major analyses were carried out on the fish samples. These were initial and final proximate analyses for each of the processing methods, chemical analysis microbial Analysis organoleptic assessment.

Proximate analysis: Proximate analysis[15] was carried out on each sample to determine the following using A.O.A.C.[15] methods moisture content, fat content, crude protein, crude fiber ash content, Nitrogen- Free-Extract (N.F.E)

Anova: For initial and final approximate analysis
Design: Completely Randomized Design (CRD)

Source of variation	d.f	s.s	m.s	Fcal	Ftab (0.05)
Treatment	5	11665.667	2333.133	0.030	2.34
Error	36	2585.792	71.827		
Total	41	4530.07			

$d.f_{0.05}$ (36;50) = 2.34, Not significant (p>0.05)

Chemical analysis: The following chemical analysis was done on the fish samples using the method as described by Pearson[16] method peroxide value, Free Fatty Acids and Total volatile base-Nitrogen (TVB-N)

Anova: For chemical parameter with length of storage
Design: Completely Randomized Design (CRD)

Source of variation	d.f	s.s	m.s	Fcal	Ftab (0.05)
Treatment	2	31.674	15.837	8.41	4.79
Error	63	118.613	1.883		
Total	65	150.287			

d.f 0.05 (63.2), Significant (p<0.05)

Microbial analysis: The microbial analysis of fresh, cold smoked, hot smoked and oven dried *Tilapia nilotica* was done by isolation, identification and counting of the micro-organism using the methods as described by Lyne[17,18].

Anova: For organoleptic assessment with length of storage
Design: Completely Randomized Design (CRD)

Source of variation	d.f	s.s	m.s	Fcal	Ftab (0.05)
Treatment	3	14.238	4.746	35.44	3.78
Error	80	10.72	0.134		
Total	83	24.988			

$d.f_{0.05}$ (83.3) = 3.78 Significant ($p<0.05$)

Organoleptic assessment: The organoleptic assessment of the processed fish was carried out by a taste panel, consisting of five students from the Department of Food Technology, University of Ibadan. The panel members were briefly trained on the organoleptic assessment of processed fish. Samples were taken randomly from the containers A, B and C, which were stored in the freezer (-25°C) and placed in three different white plates also labeled A, B, C, on a Table.

Anova: For microbial analysis with length of storage
Design: Completely Randomized Design (CRD)

Source of variation	d.f	s.s	m.s	Fcal	Ftab (0.05)
Treatment	8	2378.32	297.27	5.62	2.66
Error	81	4281.78	52.86		
Total	89	6660.10			

$d.f_{0.05}(81,8)$=2.66Significant

Each panel member was given distilled water to wash their hand and mouth to avoid any carry over of taste or contaminations. Assessment was carried out organoleptically and scored by each panel member based on a scoring system that contained measurements of certain parameter (appearance, taste, odour and texture) on graded scores ranging from 1-7.

RESULTS

In regards to the processed fish products, the cold smoked fish products had the highest final moisture content of 30.67% and the highest final crude protein content of 39.90% (Table 1), These values were closely followed by the hot smoked products, having a final moisture content of 29.09% and a final crude protein content of 38.08%. The proximate analysis and microbial analysis of fresh, cold smoked, hot smoked and oven dried *Tilapia nilotica is* represented in Table 1.

The proximate analysis of the cold smoked fish products had he highest final moisture content of 30.67% and highest final crude protein of 39.90%, this is closely followed by values of the final smoked products 29.09%(moisture constant) and 38.08% (crude protein), respectively.

Table 2 showed the cold smoked products recording the highest P.V of 4.36 meq kg^{-1} at the 12th week, FFA 7.94% and TVBN mgN/100 gm fish 3.65, This is followed by values for oven dried 4.10 meq kg^{-1}, 7.85% and 3.20 TVBNmgN/100 gm fish, while the hot smoked fish products recorded the least 3.90 meq kg^{-1}, 7.25% and 3.02 TVBN mgN/100 gm fish

The organoleptic assessment results followed a similar pattern with the chemical analysis result with the hot smoked product showing the best quality assessment out of the 3 processed product as shown in Table 3.

Table 4 shows the highest bacteria count was recorded in the cold smoked products in the first 8 weeks, oven dried fish products the first 4 weeks, while no bacteria specie were detected in the hot smoked products throughout the 12 weeks of cold storage at -25°C.

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DISCUSSION

Proximate analysis: As shown in Table 1, there was no significant difference ($p>0.05$) between the values of fresh, initial processed and final processed fish for the proximate analyses. Cold smoked samples had the highest moisture content in comparison with the moisture content of hot smoked and oven dried samples. This is because the cold smoked fish is not fully dehydrated.

Also, the crude protein of cold smoked was higher than that of the hot smoked and oven dried samples, which is probably because cold smoking has not condensed the protein and fat enough because of its high moisture content.

Chemical analysis: From Table 2, it can be seen that there was a significant difference ($p<0.05$) for all measured parameters (P.V., F.F.A., T.V.B-N). This implies that the chemical composition of the samples changed significant with storage time. It also means that the cold storage medium does not stop the spoilage of fish although it may slow down the rate of deterioration. At the second week the values of, P.V., F.F.A. and T.V.B-N. Significantly increased ($p<0.05$) for the 3 processed samples as shown in Table 2.

After two days of the experiment, cold smoked fish had the highest P.V .of 4.36 meq kg^{-1} and T.V.B. -N of 3.65 mgN 100 gm^{-1} fish while hot smoked fish product had the lowest P.V of 3.90 meq kg^{-1} and T.V.B.-N of 3.02 mgN100gm^{-1}. According to Pearson [19]who stated that white fish can be considered as fresh if the amount of

Table 1: Initial and final proximate analysis of fresh, cold smoked, hot smoked and oven dried *Tilapia nilotica* under cold storage

	Cold smoked		Hot smoked		Oven	Dried
Parameter	Fresh	Initial	Final	Initial	Final	Initial	Final
Moisture content (%)	34.77	29.74	30.67	27.45	29.09	26.60	28.65
Ash content (%)	10.34	18.85	16.51	17.70	15.49	20.18	16.06
Fat content (%)	10.85	17.36	9.72	11.30	12.45	11.97	12.75
Crude protein (%)	39.66	40.83	39.90	38.91	38.08	37.18	37.95
Crude Fiber (%)	1.31	1.42	1.19	1.23	1.31	1.16	1.13
N.F.E. (%)	3.07	2.10	2.01	3.40	3.60	2.92	3.46

Table 2: Chemical Analysis (P.V., F.F.A. and TVB-N) of fresh, cold smoked, hot smoked and oven dried *Tilapia niloticus*

		Cold smoked							Hot smoked							Oven dried						
Parameter	Fresh	0 wk	2nd wk	4th wk	6th wk	8th wk	10th wk	12th wk	0 wk	2nd wk	4th wk	6th wk	8th wk	10th wk	12th wk	0 wk	2nd wk	4th wk	6th wk	8th wk	10th wk	12th wk
P.V. (meq kg^{-1})	2.65	2.66	3.20	3.68	3.82	4.23	4.07	4.36	2.55	3.02	3.40	3.46	3.46	3.86	3.90	2.78	3.09	3.21	3.35	3.67	4.00	4.10
F.F.A. (%)	3.25	3.75	4.25	4.37	4.62	4.75	6.16	7.94	3.52	5.62	5.87	6.23	6.56	7.26	7.25	3.46	5.22	5.42	5.86	6.21	6.87	7.85
TVB-N (mgN/ 100 gm fish)	1.68	1.68	2.35	2.43	2.79	2.84	3.08	3.65	1.72	2.01	2.14	2.32	2.36	2.69	3.02	1.85	2.16	2.12	2.21	2.40	2.87	3.20

Table 3: Organoleptic assessment of cold smoked, hot smoked and oven dried *Tilapia niloticus* under cold storage

	Cold smoked								Hot smoked								Oven dried							
Parameter	0 wk	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk	Mean	0 wk	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk	Mean	0 wk	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk	mean
Appearance	5	4	4	4	4	3	3	3.57	5	5	5	4	4	4	4	4.43	5	5	5	4	4	4	4	4.43
Taste	5	5	5	5	4	4	3	4.43	5	5	5	5	5	4	4	4.70	5	5	5	5	4	4	4	4.57
Oduor	5	4	4	4	4	3	3	3.60	5	5	5	4	4	4	4	4.43	5	5	5	4	4	4	4	4.43
Texture	5	5	4	4	4	4	3	4.12	5	5	5	5	5	4	4	4.71	5	5	5	5	4	4	4	4.57
				Mean				3.93				Mean				4.57				Mean				4.50

Key- 1-Extremely unacceptable, 2-Poor, 3-Unsatisfactory, 4-Fair, 5-Satisfactory, 6-Good, 7-Extremely acceptable

Table 4: Microbial analysis of fresh, cold smoked, hot smoked and oven dried *Tilapia niloticus* under cold storage

Isolated micro-organisms	fresh	Cold smoked 1st month 2nd month 3rd month	Hot smoked 1st month 2nd month 3rd month	Oven dried 1st month 2nd month 3rd month
Staphylococcus aureus (cf ug^{-1})	----	20	----	1
		20	----	----
		----	----	----
Bacillus subtilis (cf ug^{-1})	----	40	----	1
		25	----	----
		----	----	----
Pseudomonas aureginosa (cf ug^{-1})	----	30	----	----
		----	----	----
		----	----	----
Micrococus acidiophilus (cf ug^{-1})	----	35	----	----
		5	----	----
		----	----	----
Bacillus furimus (cf ug^{-1})	----	25	----	----
		5	----	----
		----	----	----
Aspergillus terraus (cf ug^{-1})	----	----	----	----
		----	----	----
		----	----	----
Aspergillus ochraceus (cf ug^{-1})	----	1	----	----
		----	----	----
		----	----	----
Fusarium oxysporum (cf ug^{-1})	----	1	----	----
		----	----	----
		----	----	----
Sacchomyces sp. (cf ug^{-1})	8	----	----	2
		----	----	----
		----	----	----
Total (cf ug^{-1})	8	125	----	4
		55	----	----
		----	----	----

T.V.B-N. Is less than 20 mgN100g^{-1} fish and P.V values are less than 40 meq kg^{-1} of fish and From Table 2 it can be seen that the highest T.V.B.-N and P.V values fell with-in the range for fresh fish This implies that after two days, the three processed samples still fell with-in the range of fresh fish for human consumption. This was probably the case because of the cold storage temperature of -25°C for all three samples. Cold smoked fish product had the highest deterioration rate among the three processed samples.

The best processing method was hot Smoking with a P.V value of 3.90 meq kg^{-1}, F.F.A value of 7.25% and T.V.B.-N value of 3.02 mgN100 gm^{-1}, followed by oven drying with P.V value of 4.10 meq kg^{-1}, F.F.A value of 7.85% and T.V.B.-N value of 3.20 mgN100 gm^{-1} and lastly cold smoked product with P.V value of 4.36 meq kg^{-1}, F.F.A value of 7.94% and T.V.B.-N value of 3.65 mgN100 gm^{-1} fish.

Organoleptic assessment: As shown in Table 3, there was significant difference ($p<0.05$) between the values. This implies that the organoleptic parameters of the processed samples changed significantly with the time of storage.

At the time zero week (i.e. at the beginning of the cold storage experiment) all the organoleptic parameter of all the samples were rated as good by all the panel members based on the grading scale. At the 2nd week of storage, appearance and odour of only cold smoked had changed from good to satisfactory, while the taste and texture were still rated as good.

Furthermore, at the 4th week of storage, all the organoleptic parameters of hot smoked and oven dried were still good. At the 8h week of storage all the organoleptic parameter of cold smoked fish products had changed from good to satisfactory levels.At ten week the of storage the odour of cold smoked has moved down from satisfactory level to just satisfactory level. At 12th week of storage only texture of hot smoked and oven dried were still good while other parameters has moved down to satisfactory level. Also at week 12 odour of cold smoked samples had moved from satisfactory level to just satisfactory based on the scale of grading. At the end of the experiment (12 weeks)hot smoked samples had average organoleptic score of 4.57, followed by oven dried samples of 4.50 and lastly cold smoked 3.93. The best processing method is hot smoking followed by oven drying, while cold smoking resulted in the least acceptable fish products.

Microbial analysis: As shown in Table 4, there were significant differences ($p<0.05$) between the values of the bacterial count. This indicated that the microbial composition of the fresh, cold smoked, hot smoked and oven dried *Tilapia nilotic*a samples changed with time of storage at -25°C.

At the beginning of the storage experiment, bacteria were detected on the cold smoked product. Some of the bacterial detected were *Staphylococus aureus* (20 cfu g^{-1}) *Bacillus subtilis* (40 cfu g^{-1}), *Pseudomonus aureginosa* (30 cfu g^{-1}) and *Micrococus acidophilus* (35 cfu g^{-1}).

Also, at the beginning of the storage experiment, the fresh fish did not have any bacteria on it, except fungi *Saechonyces* sp. (8 cfu g^{-1}). Bacteria could be detected on the oven-dried samples up to the 4th week, since the moisture content of these samples were probably not fully converted to ice. However, bacterial species were still noticed until the 8th week in the cold smoked sample.

The best processing method was hot smoking with zero microbial counts. Hot smoked product had no microbial count during cold storage throughout the 12-week experimental period. This is followed by oven dried *Tilapia nilotica*, which showed least bacterial count of 2 cfu g^{-1} until 4 weeks of storage. The major bacteria detected were *Staphylococus aureus* (1 cfu g^{-1}) and *Bacillus subtilis* (1 cfu g^{-1}).

The processing method which resulted in the least satisfactory products, was the cold smoking process, with bacterial count of 150 cfu g^{-1} and bacterial species were detected up till 8 weeks of storage.. However, during the last four weeks of the storage experiment, no traces of bacterial growth for samples of fish from the three processing methods could be found. The prominent bacterial species in the cold smoked samples were *Staphylococus aureus* (20 cfu g^{-1}), *Bacillus subtilis* (40 cfu g^{-1}), *Pseudomonus aureginosa* (30 cfu g^{-1}) and *Micrococus acidophilus* (35 cfu g^{-1}).

CONCLUSION

Generally, at the end of the 3rd month (12 weeks) of the storage experiment, little deterioration has taken place in the samples. This was due to the fact that the storage medium was good enough to minimize the rate of loss of quality from the processed samples. It can be concluded that the best processing method was hot smoking, followed by oven drying and finally cold smoking. Hence, cold storage of processed fish products, especially at very low temperatures, will drastically reduce the rate of spoilage and also prolong the shelf life of the processed products.

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