THE EFFECTS OF PRESERVATION METHODS ON PROXIMATE COMPOSITION, INSECT INFESTATION AND ORGANOLEPTIC PROPERTIES OF Heterobranchus longifilis, Heterotis niloticus AND Chrysichthys nigrodigitatus

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ABSTRACT

A study to evaluate the effects of insecticide, Actellic 25 EC and salt solutions on proximate composition, preservation of organoleptic properties and reduction of insect infestation on traditionally smoked dried fish samples was carried out using three freshwater fishes, Heterobranchus longifilis, Heterotis niloticus and Chrysichthys nigrodigitatus. The various dehydration and smoking treatments had effect on the proximate composition. The highest moisture contents were recorded in the batch I (fresh) fish pieces, while the lowest moisture content occurred amongst the Actellic dehydrated and smoked dried fish pieces. The fat content of fish pieces dehydrated and smoked dried showed that the non dehydrated and non smoked dried fish pieces (fresh fish) had the highest fat content. The highest fibre content was recorded in the batch I (fresh fish) fish species and the lowest was recorded among the fish pieces dehydrated in salt solution before smoke drying. The protein content of fish pieces variously dehydrated and smoked dried revealed that the Actellic 25 EC dehydrated smoked dried fish pieces had the highest protein content while the lowest protein contents were recorded among the fresh fish pieces not dehydrated either in salt and/or Actellic 25 EC solutions. The highest carbohydrate content was recorded in the batch I (fresh fish) while the lowest occurred among Actellic 25 EC dehydrated smoked dried fish pieces. Two insects, Dermestes sp and Necrobia sp were identified to attack dehydrated and smoked dried fishes. The smoked dried fishes had comparatively higher insect attack than the salted and / or Actellic dehydrated smoked dried fish pieces. Fish pieces preserved with Actellic had the overall best organoleptic properties while acceptability of the dried fish was best for salted smoked dried fish pieces. The relevance of this study to humanity is discussed.

Key words: Deltamethrin, Freshwater Fish, Fish salting, Fish Smoking, Fish Storage, Chemical Properties, Organoleptic Properties

INTRODUCTION

Fish supplies a significant part of the protein nutrient of animal origin in the diet of man. From the nutritional point of view, fish is one of the most important animal proteins available in many of the less developed countries (James, 1984). FAO (1985) estimated that 53% of the world's fish harvest by developing nations is consumed locally. Unfortunately, most of these catches are lost because of lack of adequate technology to prevent post harvest losses in the third word countries (Osuji, 1976).

In Nigeria, available data show that the artisanal fishers contribute more than 95% of the local fish production and over 50% of the total fish supply (Eyo, 1992). Nwuba (1997)

established that 80% of the artisanal fishers were women. Thus indicating the predominant role women play in post harvest handling, processing and preservation of fish.

The agents of spoilage include insects, bacteria, fungi and autolytic enzymes. These agents operate under certain optimum conditions. This paper is concerned with the effect of salt and Actellic 25 EC solution on the shelf life and organoleptic properties of smoke cured *Heterobranchus longifilis, Heterotis niloticus* and *Chrysichthyes nigrodigitatus*, against insect attack. These fish species are of high commercial value in Nigeria. Actellic 25 EC is used because of it recommendation (FAO, 1985).

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Fish preservation is a process of keeping the fish close to its fresh state by minimizing changes in its physical appearance, taste and smell. The prevailing methods of preserving fish in Nigeria are still traditional curing of fish by sun-drying, smoking with or without presalt treatment or a combination of these methods (Awoyemi, 1990; Akande and King, 1997).

MATERIALS AND METHODS

Fish Procurement: A total of 864 fish pieces of about 96.84 gm average weight of fish samples – *H. longifilis, H. niloticus* and *C. nigrodigitatus* were used in this study. The fish samples were procured fresh from landing sites at Mariner water side and nearby Ose market, all at Onitsha bank of the river Niger.

Fish Processing: The fish samples were cut into uniform pieces as much as possible and divided into four batches. This is important for valid conclusions to be drawn from reliable results. Each batch contained 216 fish pieces made up of each of the three fish species.

Fish samples in batch I (fresh specimen) served as control for mineral and proximate analyses. Fish samples in batch II were traditional smoke dried and stored. Fish samples in batches III and IV were either salted or treated in 0.03 % Actellic 25 EC solution before smoke drying and storage.

25.4 % salt solution was prepared by dissolving 2.54 grams of salt in 10 litres of distilled water. 0.03 % Actellic 25 EC solution was prepared by dilution of 12.00 ml of Actellic 25 EC in 10 litres of distilled water. Batches III and IV fish pieces were dehydrated in either salt or Actellic 25 EC solutions respectively before smoke drying.

Batch III fish pieces were washed and soaked in 10 litres of brine solution for 3 hours, removed and soaked in distilled water for 15 minutes. They were subsequently drained and smoke-dried for 12 hours, wrapped in paper and stored in bamboo basket for 12 weeks under tropical ambient condition. Batch IV fish pieces were washed and soaked in 10 litres of Actellic solution for 15 seconds, drained, smoke-dried for 6 hours, sprayed with 0.5 litre of the solution, sun-dried for 3 hours, wrapper in paper and stored in bamboo basket for 12 weeks under tropical ambient condition.

To ensure that the fish samples did not deplete the active ingredients in the individual solution, 10 litres of each preservative solution

was used to ensure that all fish pieces were totally submerged in the solution.

Proximate Analysis: Specimens were collected from batch I - IV for proximate analysis to determine crude protein, fats, fibre, ash and carbohydrate values (AOAC, 1980).

Insect Infestation: Visual observation, collection and counts of all insects at the end of the storage period were done. All insects collected were preserved and identified to their species level.

Organoleptic Analysis: A panel of 10 independent judges was arranged to examine, tastes and score fish pieces from batch II - IV for odour, colour, texture and acceptability. A 9 point range score card was used thus: (1 = extremely -ve, 2 = very -ve, 3 moderately -ve, 4 = slightly -ve, 5 = intermediate 6 = slightly + ve; 7 moderately + ve; 8 = very + ve and 9 = extremely + ve.

Statistical Analysis: The proximate values arising from fish pieces in batches I-IV were analyses for their range values, mean and standard errors. F-LSD was used to separate treatment means (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The results on the proximate composition of the fish pieces from the three freshwater fish variously dehydrated and smoked dried are presented on Table 1. The highest moisture content was recorded in the batch I (fresh fish) fish species thus; H. longifilis - 70.01 + 0.027, H. niloticus – 69.29 \pm 0.053 and C. nigrodigitatus - 68.06 <u>+</u> 0.014. The lowest moisture content were recorded among the fish pieces dehydrated in Actellic 25 EC before smoke drying thus; H. longifilis - 8.89 ± 0.034, H. niloticus – 10.11 ± 0.025 and C. nigrodigitatus - 16.62 ± 0.083. Other moisture content values were thus; H. longifilis - 10.34 + 0.071, H. niloticus - 14.64 ± 0.034 and C. nigrodigitatus – 29.62 \pm 0.033 for salt dehydrated and smoked dried and H. longifilis - 20.41 ± 0.092 , H. niloticus – 18.64 ± 0.060 and C. nigrodigitatus - 19.63 + 0.046 for smoked dried fish pieces respectively. Akande and King (1997) reported similar ranges in moisture contents of West Africa sardines Sardinella maderensis using Actellic 50 EC solution.

The ash content of fish pieces variously dehydrated and smoked dried revealed that the

Table 1: Percentage proximate composition of pieces of three freshwater fish species variously dehydrated and smoked-dried

	Fresh			Smoke-dried			Salted & Smoked-dried			Actellic & Smoked dried		
	Heterobranchus Iongifilis	Heterotis niloticus	Chrysichthys nigrodigitatus									
Moisture	iongimis	moticus	mgrouigitatus	iongiins	moncus	nigrouighatus	iongiinis	moticus	mgrouighatus	iongiins	moticus	mgrouigitatus
Range	69.90 - 70.11	69.03 - 69.12	68.02 - 68.12	20.11 - 20.64	18.61 - 18.99	19.54 - 19.80	10.12 - 10.62	14.55 - 14.76	29.50 - 29.70	8.79 - 9.0	10.0 - 10.17	16.3 - 16.9
Mean	70.01	69.29	68.06	20.41	18.64	19.63	10.34	14.64	29.62	8.89	10.11	16.62
SE	0.027	0.053	0.014	0.092	0.06	0.046	0.071	0.034	0.033	0.034	0.025	0.083
Ash												
Range	1.0 - 1.2	0.99 - 1.20	0.09 - 1.10	6.13 - 6.15	6.89 - 6.91	10.00 - 12.00	14.98 - 15.22	4.7 - 5.3	4.80 - 5.12	5.9 - 6.03	3.95 - 4.05	2.96 - 3.14
Mean	1.1.00	1.03	0.983	6.15	6.9.00	11.00	15.03	5.02	5.01	5.99	4.00	3.03
SE	0.036	0.033	0.031	0.005	0.004	0.365	0.039	0.079	0.046	0.019	0.013	0.03
Fat												
Range	13.00 - 32.00	23.09 - 24.01	11.00 - 12.00	8.00 - 10.00	16.16 - 16.31	9.55 - 11.00	15.14 - 15.99	21. 00 - 21.80	13.00 - 13.45	3.49 - 3.68	3.0 - 3.10	2.99 - 3.01
Mean	22.80	23.69	11.28	9.16	16.11	10.07	15.72	21.31	13.22	3.60	3.03	3.00
SE	4.10	0.143	0.153	0.306	0.053	0.199	0.121	0.113	0.074	0.025	0.015	0.003
Fibre												
Range	0.40 - 0.45	1.40 - 1.56	1.00 - 1.10	1.00 - 1.01	1.00 - 1.02	0.18 - 0.25	0.08.0.1	0.30 - 0.33	0.24 - 0.27	0.33 - 0.37	0.58 - 0.61	0.28 - 0.33
Mean	0.428	1.51	1.04	1.00	1.01	0.207	0.092	0.317	0.253	0.35	0.602	0.30
SE	0.007	0.024	0.015	0.002	0.004	0.01	0.003	0.005	0.004	0.007	0.005	0.007
Protein												
Range	15.36 - 15.55	10.66 -1.07	17.72 - 17.78	61.11 - 61.22	45.33 - 54.34	54.27 - 54.31	55.30 - 55.42	55.22 - 55.40	40.10 - 40.20	50.31 - 50.55	58.36 - 58.90	67.23 - 67.44
Mean	15.46	10.68	17.75	61.15	52.83	54.29	55.36	55.34	40.15	50.42	58.64	67.32
SE	0.03	0.006	0.01	0.016	1.50	0.006	0.021	0.026	0.017	0.035	0.07	0.028
Carbohyd	rate											
Range	9.22 - 9.30	14.00 - 14.07	8.77 - 8.99	3.14 - 3.18	3.44 - 3.46	3.80 - 4.00	3.33 - 3.36	3.22 - 3.44	12.22 - 12.33	2.15 - 2.20	2.66 - 2.80	1.40 - 1.47
Mean	9.25	14.04	8.89	3.16	3.45	3.93	3.34	3.33	12.29	2.18	2.71	1.44
SE	0.012	0.01	0.03	0.006	0.004	0.034	0.005	0.028	0.016	0.007	0.022	0.01

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non dehydrated but smoked dried fish pieces had the highest ash content thus; H. longifilis - 6.15 ± 0.005 , H. niloticus – 6.90 ± 0.004 and C. $nigrodigitatus - 11.00 \pm 0.365$. The lowest ash contents were recorded among the fresh fish pieces not dehydrated either in salt and/or Actellic 25 EC solutions and not smoked dried thus; H. longifilis – 1.10 \pm 0.036, H. niloticus – 1.03 + 0.033 and *C. nigrodigitatus* - 0.983 + 0.031. Other ash content values recorded in this study were thus; H. longifilis - 15.03 + 0.039, H. niloticus – 5.02 ± 0.079 and C. nigrodigitatus - 5.10 ± 0.046 for salt dehydrated and smoked dried and H. longifilis - 5.99 + 0.019, H. niloticus - 4.0 ± 0.013 and C. nigrodigitatus -3.03 + 0.030 for Actellic 25 EC dehydrated smoked dried fish pieces. Our result is not in variances with Akande and King (1997) who observed almost similar range values in ash contents of West Africa Sardinella maderensis dehydrated with Actellic 50 EC before smoked drying and Nwuba (2002) who reported similar range values for three freshwater fishes dehydrated with salt before smoke drying.

The fat content of fish pieces dehydrated and smoked dried showed that the non dehydrated and non smoked dried fish pieces (fresh fish) had the highest fat content thus; *H. longifilis* – 22.80 <u>+</u> 4.10, *H. niloticus* – 23.69 + 0.143 and *C. nigrodigitatus* - 11.28 + 0.153. The lowest fat contents were recorded among the fresh fish pieces dehydrated in Actellic 25 EC solution and smoked dried thus; H. longifilis - 3.60 ± 0.025, H. niloticus - 3.03 ± 0.015 and *C. nigrodigitatus* – 3.0 \pm 0.003. Other fat content values recorded in this study were thus; H. longifilis - 9.16 + 0.306, H. niloticus -16.11 \pm 0.053 and *C. nigrodigitatus* – 10.07 \pm 0.199 for smoked dried fish pieces and H. longifilis - 15.72 + 0.121, H. niloticus - 21.31 + 0.113 and *C. nigrodigitatus* – 13.22 + 0.074 for salt dehydrated smoked dried fish pieces (Table 1). Our result was not in variances with Akande and King (1997) who reported fat contents in West Africa Sardinella maderensis dehydrated with Actellic 50 EC before smoked drying to be within the experimental range values of this study.

The results on the fibre composition of the fish pieces from the three freshwater fish variously dehydrated and smoked dried are presented on Table 1. The highest fibre content was recorded in the batch I (fresh fish) fish species thus; *H. longifilis* – 0.428 \pm 0.007, *H. niloticus* – 1.51 \pm 0.024 and *C. nigrodigitatus* – 1.04 \pm 0.015. The lowest fibre content were recorded among the fish pieces dehydrated in

salt solution before smoke drying thus; $H.longifilis - 0.092 \pm 0.003$, $H.loloticus - 0.317 \pm 0.005$ and $C.loticus - 0.253 \pm 0.004$. Other fibre content values were thus; $H.longifilis - 0.35 \pm 0.007$, $H.loloticus - 0.602 \pm 0.005$ and $C.loticus - 0.30 \pm 0.007$ for Actellic dehydrated and smoked dried and $H.longifilis - 1.00 \pm 0.002$, $H.loticus - 1.01 \pm 0.004$ and $C.loticus - 0.207 \pm 0.01$ for smoked dried fish pieces. Nwuba (2002) reported similar range values for three freshwater fishes dehydrated with salt before smoke drying.

The protein content of fish pieces variously dehydrated and smoked dried revealed that the Actellic 25 EC dehydrated smoked dried fish pieces had the highest protein content thus; H. longifilis - 50.42 <u>+</u> 0.035, H. niloticus - 58.64 ± 0.07 and *C. nigrodigitatus* – $67.32 \pm$ 0.028. The lowest protein contents were recorded among the fresh fish pieces not dehydrated either in salt and/or Actellic 25 EC solutions and not smoked dried thus; H. longifilis - 15.46 + 0.03, H. niloticus - 10.68 + 0.006 and *C. nigrodigitatus* – 17.75 \pm 0.01. Other protein content values recorded in this study were thus; H. longifilis - 55.36 + 0.021, H. niloticus - 55.34 \pm 0.026 and C. $nigrodigitatus - 40.15 \pm 0.017$ for salt dehydrated and smoked dried and H. longifilis - 61.15 ± 0.016 , *H. niloticus* – 52.83 ± 1.50 and C. nigrodigitatus – 54.29 \pm 0.006 for non dehydrated but smoked dried fish pieces. The protein content of batch I fish pieces differed significantly from batches II to IV (P > 0.05). Our result is not in variances with Akande and King (1997) for West Africa Sardinella maderensis dehydrated with Actellic 50 EC before smoked drying and Nwuba (2002) for three freshwater fishes species variously dehydrated before smoke drying.

The results on the carbohydrate composition of fish pieces from either H. longifilis, H. niloticus or C. nigrodigitatus variously dehydrated and smoked dried are presented on Table 1. The highest carbohydrate content was recorded in the batch I (fresh fish) fish species thus; H. longifilis - 9.25 + 0.012, H. $niloticus - 14.04 \pm 0.01$ and C. nigrodigitatus - 8.89 ± 0.03 . The lowest carbohydrate content were recorded among batch IV fish pieces, dehydrated in Actellic 25 EC before smoke drying thus; H. longifilis - 2.18 ± 0.007 , H. niloticus - 2.71 + 0.022 and C. nigrodigitatus -1.44 + 0.01. Other carbohydrate content values were thus; H. longifilis - 3.34 + 0.005, H. niloticus - 3.33 + 0.028 and C. nigrodigitatus -

Table 2: Insect infestation of pieces of three freshwater fish species variously dehydrated and smoked-dried

		Fresh		Smoke-dried			
	Heterobranchus Iongifilis	Heterotis niloticus	Chrysichthys nigrodigitatus	Heterobranchus Iongifilis	Heterotis niloticus	Chrysichthys nigrodigitatus	
Dermestes sp							
Range	0.00	0.00	0.00	10.00 - 25.00	60.00 - 72.00	55.00 - 63.00	
Mean	0.00	0.00	0.00	17.00	66.00	59.00	
SE	0.00	0.00	0.00	2.00	0.50	1.05	
Necrobia sp							
Range	0.00	0.00	0.00	32.00 - 44.00	61.00 - 74.00	55 - 61	
Mean	0.00	0.00	0.00	36.00	66.00	59.00	
SE	0.00	0.00	0.00	3.60	2.00	2.50	

	Salte	d & Smoked-dr	ried	Actellic & Smoked dried				
	Heterobranchus Iongifilis	Heterotis niloticus	Chrysichthys nigrodigitatus	Heterobranchus Iongifilis	Heterotis niloticus	Chrysichthys nigrodigitatus		
Dermestes sp						_		
Range	20.00 - 24.00	22.00 - 28.00	12.00 - 18.00	12.00 - 15.00	6.00 - 11.00	6.00 - 9.00		
Mean	22.00	25.00	17.00	13.00	5.50	7.00		
SE	1.00	2.00	2.50	1.00	2.50	1.50		
Necrobia sp								
Range	22.00 - 25.00	15.00 - 17.00	12.00 - 18.00	10.00 - 13.00	6.00 - 9.00	4.00 - 8.00		
Mean	23.00	16.00	16.00	12.00	7.00	6.00		
SE	1.00	1.00	2.00	1.00	1.50	2.00		

12.29 \pm 0.016 for salt dehydrated and smoked dried and *H. longifilis* – 3.16 \pm 0.006, *H. niloticus* – 3.45 \pm 0.004 and *C. nigrodigitatus* – 3.93 \pm 0.034 for smoked dried fish pieces. The protein content of batch IV fish pieces differed significantly from batches I to III (P > 0.05). Similar ranges values in carbohydrate contents have been reported for West Africa sardines *Sardinella maderensis* (Akande and King, 1997).

Two insects, Dermestes sp and Necrobia sp were identified to attack dehydrated and smoked dried fishes. The smoked dried fishes had comparatively higher Dermestes sp attack when compared to either salt and / or Actellic dehydrated smoked dried fish pieces thus; H. longifilis - 17.00 + 2.00, H. niloticus - 66.00 + 0.50 and C. nigrodigitatus -59.00 + 1.05 (Table 2). Actellic treated fish pieces had the least number of Dermestes thus; H. longifilis - 13.00 + 1.00, H. niloticus - 5.5 + 2.50 and *C. nigrodigitatus* – 7.00 \pm 1.50. Similarly, the smoked dried fish pieces had higher Necrobia sp attack when compared to either salt and / or Actellic dehydrated smoked dried fish pieces thus; H. longifilis - 36.00 + 3.60, *H. niloticus* – 66.00 \pm 2.00 and *C.* nigrodigitatus - 59.00 <u>+</u> 2.50 (Table 2). Actellic dehydrated and smoked dried fish pieces had the least number of *Necrobia* sp *thus; H. longifilis* – 12.00 ± 1.00 , *H. niloticus* – 7.00 ± 1.50 and *C. nigrodigitatus* – 6.00 ± 2.00 . The insect attack on Actellic treated fish pieces (batch IV fish pieces) differed significantly from fish pieces batches I to III (P > 0.05). Esser *et al* (1990), reported low insect infestation arising from the use of insecticides to protect salt-dried marine catfish during processing and storage.

The results of the sensory evaluation, texture and taste are shown in Figure 1. The texture of the dried fishes in batch IV were the best followed by those in batches III and II. The fish samples in batch I were not assayed for organoleptic properties. Evaluation of the odour showed that the fish pieces in batch II had the worse odour after storage while those stored after dehydration and smoking, batches (III and IV) had the better odour. Acceptability of the dried fish was best for those in batch III.

When all the parameters used in the judgment are put together and analyzed, the overall performance of the various treatments can be readily seen. For instance, the fish pieces preserved with Actellic had the overall best performance (6.49 ± 0.14) .

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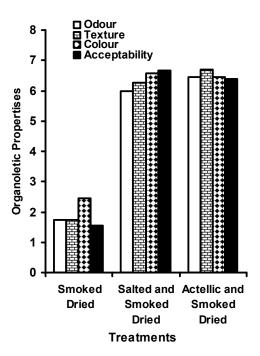


Figure 1: Organoleptic propertise of varoiusly dehydrated and smoked dried freshwater fish pieces.

This was followed closely by batch III (6.37 \pm 0.14), while the batch II fish pieces had the overall worst performance (1.87 \pm 0.14). Ikeme (1985) reported the extension of shelf life and preservation of organoleptic properties in salted smoked dried fish. The results of the study showed that for better performance of smoke dried fish, application of insecticide before smoke-drying yielded better products.

Finally, despite our results in this study, it is suggested that alternative method such as the use of natural preservatives and physical screening of processed fish from insect be used instead of chemicals. Furthermore, the recommendation of FAO/WHO safe level for food preservatives and insecticides should be strictly adhered to for health reasons.

REFERENCES

AKANDE, G. R. and KING, M. A. (1997). Control of insect infestation in smoked West Africa sardines *Sardinella maderensis* using Actellic 50 EC solution. *Nigerian Institute of Oceanography and Marine Research (NIOMR)* 1997 Annual Report, pp 16 – 17. AOAC (1980). *Official Methods of Analysis*, 11th

Edition. Association of Official Analytical Chemists, Arlington, VA 1128 pp.

- AWOYEMI, M. D. (1990). Some experiments on control of insect pest of dried fish in the Kainji Lake Basin. *Nigerian Institute of Freshwater Fisheries Research (NIFFR)* 1990 Annual Report, pp 110 120.
- ESSER, J. R., HANSON S. W. and KNOWLES, K. J. (1990). Evaluation of insecticides to protect salt-dried marine catfish from insect infestation during processing and storage. *Proceedings of Seminar in Multi-disciplinary Studies on Fish and Inshore Coastal Resources Management, Diponego University.*
- EYO, A. A. (1992). The role of fisheries technology in the development of fisheries resources in Nigeria. *Proceedings of National Conference on Lake Kainji, Nigerian Institute of Freshwater Fisheries Research (NIFFR), New Bussa, Nigeria.* pp 167 178.
- FAO (1985). World catch and Trade of Fishery product in 1984. *InfoFish Marketing Digest, No. 3:* 25.
- IKEME, A. J. (1985). Studies on brine preservation of fish and its effect on the organoleptic quality. *FAO fisheries Report, No. 329* (supplement): 136 146.
- JAMES, D. (1984). The future of fish in nutrition. *Infofish Marketing Digest No. 2*: 25.
- NWUBA, L. A. (1997). The predominant Role of the Women in fish handling Processing and preservation. *United Nations Development Programme (UNDP) sponsored training workshop on artisanal fisheries development, January 1997, Anambra State, Nigeria.* pp 10 – 16.
- NWUBA, L. A. (2002). A study on the effect of some preservatives on the Nutrient values and storage of freshwater fish: Heterobranchus longifilis, Heterotis niloticus and Chrysichthys nigrodigitatus. PhD Thesis, Department of Biological Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
- OSUJI, F. N. C. (1976). The influence of traditional handling methods on the quality of processed fish in Nigeria. *Proceedings of Conference on Handling, Processing and Marketing of Tropical Fish London. July 5 9th 1976.* pp 319 322.
- STEEL, R. G. D. and TORRIE, J. A. (1980).

 Principles and procedures of statistics: A biometrical approach. 2nd Edition.

 McGraw-Hill Book Company, New York. 633 pp.