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Chemical and Biological Assessment of *Chrysichthys furcatus* from River Niger at Cable-point, Asaba

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ABSTRACT

A life fish is of high nutritive value than otherwise the case, when invaded by bacteria hours after harvest. The study chemically and biologically assessed the comparative changes in *Chrysichthys furcatus* preserved at different temperatures. Eighteen brood-stocks were purchased from fisher-folks and conveyed to the laboratory where measurements were taken. Nine specimens were smoked with 30% moisture retention (and retained under ambient temperature) while the remaining nine specimens were preserved in the freezer at -4°C. Twenty grams tissue of each specimen was homogenized in 250 mL of 0.1% peptone water for chemical examination. The most probable number was used for counting bacteria isolates. Two samples each from smoked and refrigerated pools were examined every 24 h and on weekly basis, respectively. Five bacteria isolates were identified. Visual observation of smoked fish after 48 h revealed that spoilage had set in. Enumeration of bacteria revealed increases in smoked samples and reduction in refrigerated ones. Bacteria isolated from the fish immediately after harvest was 116×10^9 CFU g^{-1}. It increased to 268×10^9 CFU g^{-1} after 4 days of smoked preservation and reduced to 64×10^9 CFU g^{-1} after four weeks of freezer preservation. Increases were also observed in chemical parameters. For instance, total volatile nitrogen at harvest 15.3 mg N/100 g, increased to 35.2 mg N/100 g after four days and to 26.3 mg N/100 g after four weeks preservation. Reduced chemical parameters and decreased bacteria isolates were associated with freezer preservation. This preservation method increased shelf life and should be recommended to consumers.

Key words: Cold smoking, freezing, bacteria load, preservation, *Chrysichthys furcatus*, shelf life

INTRODUCTION

In Nigeria, the practice of holding harvested fish for long periods between catching and landing in open canoes without any form of preservation is worrisome. This action often results in quality changes as well as contamination of the fish by bacteria from the holding materials (Odoli, 2006). This unwholesome practice does not conform to simple hygiene, in keeping with the public health standards of fish consumption (Eyo, 2001).

The preservation of harvested fish was therefore of utmost importance since fish was highly susceptible to deterioration immediately after harvest (Okonta and Ekelemu, 2005). Smoked fish will remain a traditional part of diet of a large section of the world's population (Foline *et al*., 2011). Smoking and freezing are the most common methods used by fish processors in Nigeria (Saliu, 2008). Processing has been reported to affect the chemical composition of the fish processed.

Several workers have linked the availability of vital nutrients in fish to their method of storage (Saliu, 2008). Fish meat contains significantly low lipids and higher water than beef or chicken and is favoured over other white or red meat (Nestel, 2000).

Bacteria are ubiquitous, infesting the fish when the conditions are right and resulting in losses to fish mongers and consumers. Fish spoilage has been the result of series of complicated changes that makes it unfit for human consumption. Fishermen lose income when fishes are not adequately preserved (Clucas, 1985; Eyo, 2001). To manage and overcome fish spoilage, preservation which is a branch of manufacturing has been used to transform raw fish into nutritious and safe products for human consumption.

The present study identified the bacteria causing spoilage in frozen and cold smoked specimens of *Chrysichthys furcatus*. It investigated the chemical changes taking place in cold smoked and frozen specimens. Results of biological and chemical test, in addition to physical observations were used to determine the shelf life of the specimens of study. The study also investigated the influence of temperature abuse on the shelf life of *Chrysichthys furcatus*, identified which of the two preservative methods better extends the shelf life of the specimens and observed changes in PVC, FFA, PV and pH. It ascertained if differences exists in the bacteria load/count of cold-smoked and frozen specimens.

The need to increase fish consumption when proper preservative methods are used for newly harvested products is re-echoed. This is in keeping with the need to improve fisheries contribution to the national economy through employment creation, foreign exchange earnings and food security support.

MATERIALS AND METHODS

Sample collection, preservation and bacteria identification: Eighteen specimens of *Chrysichthys furcatus* were purchased from fisher-folks in September, 2009 at the bank of the River Niger at Cable-point, Asaba, Delta State, Nigeria. The study lasted for one month. Two specimens were randomly selected and examined immediately after catch to ascertain their organoleptic status, bacteria loads and chemical qualities of the fish. Out of the remaining sixteen specimens eight were cold smoked with 30% of moisture retained and stored on sterile table covered with heat treated wire gauze. A pair of the specimens were randomly withdrawn from the pool and examined every 24 h for bacteria isolates and chemical changes in the fish. The other set of eight specimens were preserved in the deep freezer at a temperature of -4°C. A pair of specimen from the deep freezer were randomly picked and observed weekly for one month as stated earlier (Brock *et al*., 1984; Clucas, 1985). Processed specimens from both preservative methods were tested for total aerobic bacteria count according to Eyo (2001). Suspicious bacteria colonies were identified on the basis of colonial morphology, cultural appearance and conventional biochemical tests according to the American Public Health Association (APHA) and Food and Drug Administration (FDA) isolation methods (APHA, 1977; Brock *et al*., 1984; Lyhs *et al*., 2001).

Physical, chemical and biological examination of specimens: Physical assessment using characteristic features of skin color, odor, texture, appearance and rigidity of fillets was carried out on cold smoked specimens. Thereafter, chemical and biological assessments were conducted on the cold smoked and frozen specimens according to Abdalla (2000). For chemical assessment, twenty grams of the frozen and cold smoked fish specimens were taken separately and homogenized in 250 mL of 0.1% (w/v) peptone water before a tenfold serial dilution was prepared (Odoli, 2006). The Most Probable Number (MPN) was used for counting bacteria isolates at the Fisheries

Laboratory located in Delta State University, Asaba Campus. MacConkey and Blood agar were used for the culture of bacteria isolates. Chemical changes: in pH, Total Volatile Nitrogen (TVN), Peroxide Value (PV) and Free Fatty Acid (FFA) of cold smoked and frozen fish specimens were determined at the Nigeria Institute for Oil Palm Research (NIFOR) in Edo State. The statistical techniques used were chi-square (χ^2) and t-test analysis.

RESULTS

The colony forming units of bacteria ($\times 10^9$ CFU g^{-1}) isolated from the freshly caught specimens together with those identified from the processed specimens using both preservative methods are shown in Table 1. The Table 1 revealed that five bacteria types (*Salmonella* spp. *Vibrio cholera*, Escherichia coli, *Staphylococcus aureus* and *Proteus vulgaris*) were isolated from the specimens. *Escherichia coli* recorded the highest number (55×10^9 CFU g^{-1}) of bacteria; while the least was recorded for *Salmonella* spp. (8×10^9 CFU g^{-1}) immediately after fish harvest. In week one, *Vibrio cholera* recorded the highest number (33×10^9 CFU g^{-1}) in the frozen specimens while *Staphylococcus aureus* recorded the least value (5×10^9 CFU g^{-1}). *Proteus vulgaris* recorded the highest bacteria (71×10^9 CFU g^{-1}) isolate from the cold smoked specimens after four days. This bacterium was not isolated from the frozen specimen all through the study period. Generally, the bacteria isolates from the cold smoked specimens kept increasing by the day, whereas those isolated from the frozen specimens were on the decrease.

Table 2 revealed that the calculated value of Chi square (χ^2) which is 9.96, is higher than chi square critical value 7.82, indicating a significant difference of bacteria load between frozen and cold smoked specimens.

Table 1: Colony forming units of bacteria identified using both preservative methods

	Preservative methods and total count ($\times10^9$ CFU g^{-1}) of colony forming bacteria								
Bacteria isolates	After harvest	Frozen specimens after 1 week	Cold smoked specimens after 1 day 1	Frozen specimens after 2 weeks	Cold smoked specimens after 2 days	Frozen specimens after 3 weeks	Cold smoked specimens after 3 days	Frozen specimens after 4 weeks	Cold smoked specimens after 4 days
Salmonella spp.	8	7	19	5	23	3	37	3	44
V. cholera	40	33	44	21	43	8	30	3	28
E. coli	55	30	53	20	61	17	66	8	68
S. aureus	13	5	10	30	38	46	51	50	57
P. vulgaris	-	-	20	-	45	-	62	-	71
Total	116	75	146	76	210	74	246	64	268

Table 2: Chi-square analysis of bacteria count from frozen and cold smoked specimen of *Chrysichthys furcatus*

	Bacteria count/week using both preservative methods			Chi-square analysis	
Time (weeks/days)	Frozen fish	Cold smoked fish	Total	χ^2 calculated	χ^2 critical
14	5 (3.60)	30 (31.4)	35 (35.0)	9.96	7.82
2	30 (34.50)	300 (295.9)	330 (330.0)		
3	80 (101.40)	900 (878.6)	980 (980.0)		
4	200 (175.90)	1500 (1524.1)	1700 (1700.0)		
Total	315.00	2730.0	3045.0		

DF: 17, Level of significance = 0.05, Data in parenthesis are range values which shows bacteria from frozen fish were on decrease and from cold smoked fish were on the increase

Table 3: Proximate analysis of fish samples during the period of study

		Proximate analysis of fish specimens			
Time (days/weeks)	Methods of preservation	FFA (%)	TVN (mg/100 g)	PV (meq kg^{-1})	pH
After harvest	Fish from the river	0.90	15.3	3.7	7.90
End of day 1	Cold smoke	1.42	27.1	10.2	5.20
End of week 1	Frozen	1.20	19.2	4.1	4.80
End of day 2	Cold smoke	1.73	30.2	11.3	5.25
End of week 2	Frozen	1.88	24.1	7.2	4.90
End Of day 3	Cold smoke	2.04	32.2	13.7	5.30
End of week 3	Frozen	2.00	25.4	8.1	5.10
End of day 4	Cold smoke	2.35	35.2	14.0	5.50
End of week 4	Frozen	2.24	26.3	8.9	5.30

FFA: Free fatty acid, TVN: Total volatile nitrogen, PV: Peroxide value and pH: Hydrogen-ion-concentration

Table 4: The 't' values for TVN, PV, pH and FFA analysed from frozen and cold smoked fish specimens

	TVN				pH				PV				FFA			
Methods of preservation	X	SD	Mean diff.	t-value	X	SD	Mean diff.	t-value	X	SD	Mean diff.	t-value	X	SD	Mean diff.	t-value
Frozen	23.8	2.57	7.4	4.63	5.03	0.19	0.28	3.26	7.0	1.82	5.1	5.26	1.83	0.38	0.1	3.29
Cold smoked	31.2	2.97			5.31	0.09			12.2	1.54			1.89	0.35		

TVN: Total volatile nitrogen, pH: Hydrogen-ion-concentration, PV: Peroxide value, FFA: Free fatty acid

Table 3 revealed that the values for Free Fatty Acid (FFA), Total Volatile Nitrogen (TVN), Peroxide Value (PV) and hydrogen ion concentration (pH) analysed from the specimens at the end of the experiment increased well above the values observed immediately after fish harvest from week 1 to week 4 when freezing and cold smoke preservations were ended. The values derived from the cold smoked specimens were higher than those derived from the frozen specimens at the end of the study.

There was a significant difference between the rate of spoilage for frozen and cold smoked specimens. The cold smoked specimens exhibited higher and visibly detected spoilage indices which were not observed in frozen specimens.

The values observed following the t-test analysis for Total Volatile Nitrogen (4.63), Hydrogen-ion concentration (0.28) and Peroxide Value (5.26) are presented in Table 4. The observed t-values were greater than their critical t-values (2.45), except for Hydrogen-ion concentration whose value was less than the critical value of (0.28). It can therefore be concluded that there is a significant difference between the biochemical derivatives of frozen and cold smoked specimens of *Chrysichthys furcatus*. Results of the physical assessment carried out for the cold smoked specimen by the taste panellists revealed greater spoilage outcomes than those observed for the frozen specimens. This implies that cold smoked specimens spoilt at a faster rate compared to the frozen specimens.

DISCUSSION

The prevalence of bacteria isolates in number and kind/types between the results of the present and past researchers agrees with those in literature (Hansen *et al*., 1998; Eyo, 2001; Lyhs *et al*., 2002; Olokor *et al*., 2007).

Difficulties using total counts of bacteria was reported, Lyhs *et al.* (2002). They opined that the initial count of bacteria in freshly caught fish was variable, whereas their systematic increase at temperatures between 0 and 20°C occurred under irregular temperature storage conditions. It was also reported that there is no correlation between total count of bacteria and spoilage because not all bacteria results in spoilage (Odoli, 2006). Again there is no clear link between the organoleptic characteristic in spoilage when specific bacteria spoilage organisms are present (Olokor *et al.*, 2007).

The result of the present study revealed that *E. coli and Salmonella* species were not identified in the cold smoked specimens. However, *Staphylococcus* species was present in salted cold smoked fish due to their survival during salting and smoking processes. This observation is similar to those reported (Lyhs *et al.*, 2002) and portends risk for *Staphylococcus* species infection and intoxication in humans.

The rejection point for the colony forming unit of bacteria was 10^7-10^8 CFU g^{-1} (Hansen *et al.*, 1998). This count was lower than that observed in the present study 268×10^9 CFU g^{-1} after 4 days of preservation; while that isolated from the frozen specimens after four weeks of preservation was 64×10^9 CFU g^{-1}. The range in bacteria isolates from the frozen specimens was (30-200$\times10^9$ CFU g^{-1}) whereas that encountered from the cold smoked specimen was (30-500$\times10^9$ CFU g^{-1}). These numbers of bacteria isolates far exceeds the ideal for a healthy fish (Hansen and Huss, 1998). Bacteria isolates differs in counts and types of various fish species for various studies.

The result of the present study has shown that there is a significant difference between the bacteria load of frozen and cold smoked specimens of *Chrysichthys furcatus*. The cold smoked specimens retained high moisture content after processing, which resulted to increased bacteria activities. This observation was supported by Eyo (2001). High moisture content promoted spoilage of the fish products.

The shelf- life of the frozen fish specimen in the present study is one week and two days, while that of the cold-smoked fish was twenty-four h. This finding could be explained by the fact that when the fish was in the freezer, all bacteria activities become inactive, reduced or halted. According to Olokor *et al.* (2007), when the temperature of a fish product is reduced, bacteria activities are retarded and spoilage processes halted. The frozen specimens exhibited longer shelf-life than the cold smoked ones. Increased moisture content of the cold smoked fish enhanced spoilage hence it promoted fish spoilage under cold smoking faster than all other traditional methods of preservation including freezing.

The difference in the rate of spoilage of the two groups of specimens could be attributed to factors such as methods of fish harvest, storage temperature, moisture content of smoked fish among others (Clucas, 1985).

The following values of free fatty acids (FFA >1.5%), total volatile nitrogen (TVN >20 mg N/100 g), peroxide value (PV >5.0 Milliequivalent/kilogram (meq kg^{-1}) and pH (<6.7) indicates the safe range for a healthy fish product and an ideal state of fish for consumption (Pearson and Cox, 1976; Olokor *et al.*, 2007). According to Huss (1988) the most significant factor influencing the texture of the fish and the degree of gaping/rupture of their connective tissues are changes in pH which drastically affects the properties of the connective tissues. Values of pH observed in the specimens of study after one week and two days (frozen specimens) and after 24 h (cold-smoked specimen) revealed that spoilage had set in. Again, the values for TVN, PV and pH at the end of the first day revealed that spoilage had commenced in the cold smoked specimens.

The high prevalence of lactic acid bacteria at a high level in the present study was expected since past studies showed that such bacteria species proliferates faster (Duffes *et al.*, 1999; Martinsdottir, 2002) under ambient conditions.

It is useful conducting microbiological and chemical analyses in parallel with sensory evaluation to have supporting information about the spoilage of fish (Chytiri *et al*., 2004).

CONCLUSION

Based on the findings of the present study, it was concluded that the frozen method of preserving *Chrysichthys furcatus* was comparatively better than the cold smoked method. This is because the frozen specimens exhibited longer shelf-life than the cold smoked specimen.

RECOMMENDATIONS

In view of the present findings, the following recommendations are made:

- Cold smoking alone should not be used as the only method of preserving fish because at best it adds flavour to the fish product, allowing a shorter shelf-life compare to other methods used in preserving fish
- In order to extend the shelf-life of a cold smoked fish, intermittent sun drying and or daily smoking could be done
- Moisture content less than 10% should be maintained in cold smoked fish, in order to reduce the growth of bacteria. Cold smoked fish can also be oven-dried, refrigerated or frozen, to inhibit the growth of some spoilage bacteria
- Where electricity is made constant, refrigeration of fish would remain the best method of fish preservation. This is because it does not only keep the fish in a near fresh state, but also prevents the growth of bacteria

The result of the present study will serve as key factor in preventing post harvest losses, hence improving the overall freshness of fish landed. This will inturn increase revenue of the fisher folks in general and the Nigeria populace in particular.

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