SMOKING IMPACT ON THE MICROBIAL LOAD OF *Clarias gariepinus*

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Abstract

Effects of different smoking methods on microbial load on freshly collected freshwater mud fish, Clarias gariepinus samples from Oyo State Fisheries Department, Ibadan in South-Western Nigeria was carried out. Seventy-two C. gariepinus (505 ±0.45g and 25.5 ±1.30cm) were collected and sorted into 4 groups. 10 fish samples from groups 1, 2 and 3 were subjected to cold smoking, hot smoking and oven drying methods respectively using group 4 as standard. Samples were tested for microbial loads in triplicate at the end of 24, 48 and 72 hrs. Some of the microbes identified were; Streptococcus faecium, Proteus vulgaricus, Pseudomonas aureginiosa, Bacillus cereus; Micrococcus acidiophilus, Aerobacter aerogenes, E. Coli, Streptococcus pyrogenes, Streptococcus lactis, Bacillus subtilis, Pedio cerevisiae, Sacchomyces Sp, Fusarium oxysporum, F. compacticum, Penicillium oxalicum, P. chrysogenum, Aspergillus tamari, A. niger, Cladosporum sphacrosporum, Aspergillus terreus and Rhizopus nigericans. Others are Pseadomonas, Alteromonas, Moraxella, Acinetobacter, Flavobacterium, Cytophaga, Vibrio, Aeromonoas, Staphylococcus aureus, Samonella faecuim, P. fragii. Microbial load for cold smoked products were 72%, 66% and 38%; hot smoked products had 61%, 32% and 08%; while oven-dried had 12%, 0% and 0% at 24, 48 and 12 hrs respectively. Positive correlation r = 0.94, 0.92 and 0.51 were observed between the microbial load processing methods – oven dried, hot-smoked and cold-smoked respectively. Cold smoked had 41.63% C.P and oven-dried had 25.73% C.P. Consumers had highest preference (40%) for hot smoked, 35% for oven dried and 25% for cold smoked products.

Key Words: *Processing, storage, smoking, bacteria, Clarias gariepinus.*

Introduction

Fish is indispensable in the diet because of its high quality protein content. In spite of these merits, fresh fish spoil very readily after capture due to high ambient tropical temperature which accelerates the activities of bacteria, enzymes and chemical oxidation of fats in the fish. Oxidation is responsible for the onset of rancidity which in fatty fish could be more serious in determining the shelf life of the fish than bacterial spoilage (Eyo, 2001). Smoke-drying had been used for centuries in preserving fish, and is still widely used for this purpose among several communities in the third world where up to 70% of fish catch is smoked (Ward, 1995). In industrialized countries, however, fish smoking is done for enhancement of flavor and texture (Dillon *et al.*, 1994), often producing value added products whose preservation is achieved by other means.

Clarias gariepinus is best accepted freshwater food fish in urban countries especially in Nigeria. It is also widely cultured in Nigeria. Delay or prevention of microbial spoilage of fish may be achieved by different preservative methods which include the use of smoking and chemical preservatives. Fish can be processed as: cold smoking at a temperature not exceeding 30^{o}C, hot smoking at temperature of 80^{0}C, a condition that causes thermal denaturation of the proteins, and as hot smoke–drying (Doe, 1998). The choice determines the texture, flavor and potential uses of the fish. In order to satisfy the consumer demand, it is necessary to produce good quality and safe smoked fish. Smoked fish and shellfish products can be a source of microbial hazards including *Listeria monocytogenes*, *Salmonella spp.*, and *Clostridium botulinum* (Heinitz and Johnson, 1998).

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This study focused on the effect of different smoking methods on the nutritional composition of *Clarias gariepinus* and determines the changes in the bacterial flora load and counts associated with each processing technique over 24 to 72 hours.

Materials and Methods

Seventy-two freshly caught adults of *Clarias gariepinus* (505 ± 0.45g and 25.5 ± 1.30cm SL) were obtained from Oyo State Fisheries Department, Ibadan and preserved in a vacuum flask (50 L) filled with ice flakes and transported to the microbiology laboratory of the Institute of Agricultural Research and Training (I.A.R & T), Moor Plantation, Ibadan. The samples were stored in the laboratory Refrigerator at 0 ^{0}C, completely covered with crushed ice, without direct contact, to avoid microbial cross-contamination and washing of solute compounds. The fish were sorted into four groups (A, B, C, and D) and subjected to different processing methods: (i) cold smoking, (ii) hot smoking and (iii) oven drying methods respectively using the fresh state (group D) as a standard/ control.

(a) Cold smoking: Some samples in group A were smoked for less than 2 hours at temperature of 22 24^{o}C.

(b) Hot smoking: Some samples randomly selected from group B were smoked at temperature between 70-90^{o}C, using the method described by (Eyo, 2001) for 6 to 8 hours.

(c) Oven drying: Some samples from group C, were oven-dried at 60^{o}C for 3hours to prevent denaturation, and later at over 90^{o}C for 2 days until a constant weight was obtained**.**

Bacterial counts**:** 10 g of each fresh, cold-smoked, hot-smoked and oven-dried fish samples were separately homogenized, and 0.1% buffered peptone water was used to dislodge possible contaminants. Serial dilutions of each homogenate were prepared as described by Russel (2001). Total aerobic count, number of colony forming units per ml (cfu/ml), total coliforms, faecal coliforms, yeasts and moulds were determined as described by Refai (1979). Representative colonies of the microorganisms were gram-stained, purified and stored in nutrient agar slants at 4 ^{0}C.

Characterization of isolated microbes: Culture isolates were identified after HAGS (2002). Biochemical tests; Catalase test, motility test, endospore/indole test, methyl-red test, Vogesproskauer test, oxidase test, oxidative fermentation, utilization of sugars and carbohydrates, were carried out to characterize bacterial isolates. Characterizations employed for fungal isolates were made by the methods of colonial features, seminar characteristics at (100 x 400), microscope identification identification after HAGS (2002), and also using conventional techniques.

Proximate composition of samples from each group were analyzed in triplicate as described by A.O.A.C., (1990) for crude protein, crude fat, ash, fibre, dry matter and moisture content. Ca, P, Na, K, and Mg, Cu, Zn, and Fe were determined using atomic absorption Spectophotometer (AAS), flame photometer, colorimeter as appropriate after AOAC (1990). Analysis of variance (ANOVA) test using the Complete Randomized Block Design and DMRT were used. These tools were used in comparing the association between the storage period/exposure time in nutrient quality parameters of fresh, cold smoked, hot-smoked, and oven – dried fish samples.

Result and Discussion

Microbes detected before preservation are shown in Table 1comprising of both pathogenic and non pathogenic bacteria. The pathogenic bacteria are; *Streptococcus faecium, Proteus vulgaricus, Pseudomonas aureginiosa, Bacillus cereus*; non-pathogenic bacteria are *Micrococcus acidiophilus, Aerobacter aerogenes, E. Coli, Streptococcus pyrogenes, Streptococcus lactis, Bacillus subtilis,* and *Pedio cerevisiae.* The yeast observed was *Sacchomyces Sp*) and fungi (*Fusarium oxysporum, penicillium oxalicum, penicillium chrysogenum, Aspergillus tamari, Alternaria sp., Aspergillus niger, Cladosporum sphacrosporum, Fusarium compacticum, Aspergillus terreus* and *Rhizopus nigericans*). The commonest groups of bacteria found were the Gram-negative: *Pseadomonas, Alteromonas, Moraxella, Acinetobacter, Flavobacterium, Cytophaga, Vibrio, Aeromonoas,*

and the Gram-negative groups predominate in colder waters, while in warm waters the Gram-positive flora tends to predominate. In a related study the bacteria isolated were, in descending order of frequency of occurrence, *Achromobacter, Pseudomonas, Flavohbacterium, Sarcina, Kurthia, Lactobacillus and Streptococcus spp.* (Kiser and Backwith, 1994). *Aeromonas, Vibrio and coryneform* bacteria are also present in the fresh and cold smoked samples.

The microbial analysis of the fresh, cold smoked, hot smoked and oven dried *C. gariepinus* samples are represented on Table 2. There were significant differences ($P<0.0001$) between the values of bacteria counts and particular type/species of microbes found. At higher temperatures, yeast (*Sacchomyces Sp.*) and fungi (*Fusarium oxysporum*, *Aspergillus niger, Fusarium compacticum* and *Rhizopus nigericans*) are more resistant to the extreme heat for example oven at 80^0C.

The proximate composition of the samples in these states (fresh, cold-smoked, hot smoked) exhibited a significant variation ($P<0.0001$). The highest mean crude protein content was obtained from both fresh and cold smoked samples at Zero (0) h and 24h (CS_0 and CS_{24}) while the least is obtained from hot smoked samples at 0 h and 24h (HS_0 and HS_{24}). Furthermore, the cold-smoked samples (CS_0) at 0 hour and fresh samples of the catfish recorded the highest crude fiber content, while the least was obtained from oven-dried samples at 48 hours (OD_{48}).

Ash content of sample generally increased across the media as the temperature increased while the moisture content (MC) of sample decreased. The quality of smoked product is dependent on several factors including the quality of the fish at the time of smoking, and the nature of wood and type of smoking procedure employed. The effect of curing by smoking with respect to quality and shelf life of the product depends on the preparation of the raw material, the type of smoking, relative humidity, velocity, temperature, density, and composition of the smoke, and the time of smoking (Doe, 1998).

Conclusion

Reduction of moisture content (MC) and eradicating most microbes at an extreme temperature beyond 80^0c denature the protein composition of the fish samples. That is *Staphylococcus aureus* (pathogenic) and *Micrococcus acidiophilus* (non-pathogenic). Simultaneously, hot-smoking maintains an appreciable value of the protein content of the fish samples as an intermediary between that of the fresh/cold-smoked and the oven-dried samples.

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Table 1: Microbes detected in the fresh samples

Pathogenic bacteria	non-pathogenic bacteria	Others
Streptococcus faecium,	*Micrococcus acidiophilus,*	*Sacchomyces Sp*
Proteus vulgaricus,	*Aerobacter aerogenes,*	*Fusarium oxysporum,*
Pseudomonas aureginiosa,	*E. Coli,*	*penicillium oxalicum,*
Bacillus cereus	*Streptococcus pyrogenes,*	*penicillium chrysogenum,*
	Streptococcus lactis,	*Aspergillus tamari,*
	Bacillus subtilis,	*Alternaria sp.,*
	Pedio cerevisiae	*Aspergillus niger,*
		Cladosporum sphacrosporum,
		Fusarium compacticum,
		Aspergillus terreus
		Rhizopus nigericans

TABLE 2 - Microbes occurring in the various groups of *C. gariepinus*

Cold-smoked		Hot-smoked		Oven-dried	
S. faecium	2.74 x 10^4	*S. aureus*	0.34 x 10^4	*M acidiophilus*	1.14 x 10^4
P. vulgaricus	3.35 x 10^4	*S. faecium*	1.26 x 10^4	*A aerogenes*	1.21 x 10^4
P.aureginiosa	7.58 x 10^4	*M acidiophilus*	1.78 x 10^4	*P. florescence*	0.17 x 10^4
B. cereus	5.04 x 10^4	*P. aureginiosa*	3.64 x 10^4	*P. morganii*	0.34 x 10^4
A aerogenes	1.04 x 10^4	*B. Subtilis*	1.10 x 10^4	*S. lactis*	1.26 x 10^4
E. coli)	1.14 x 10^4	*P. microbilis*	1.86 x 10^4	*B. subtilis*	1.78 x 10^4
F. Oxysporum,	4.74 x 10^4			*B. firmus*	0.34 x 10^4
P. Oxalicum	5.56 x 10^4			*Aspergillos niger*	0.17 x 10^4
Rhizopus	7.18 x 10^4			*Sacchomyces Sp.*	0.34 x 10^4
nigericans	7.64 x 10^4			*Fusarium compacticum*	1.26 x 10^4
					1.10 x 10^4