

Full Length Research Paper

Medical implications of bio-deteriorating agents in stored fish samples in Nigeria

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The objectives of this study were to examine the physical, microbial and nutritional qualities of smoked fish during storage at room temperature. Smoked fish samples were obtained from a processing plant in Osogbo metropolis (Osun state, Nigeria) and stored at room temperature for four months. They were analyzed for insect pest activity, microbial spoilage, hydrogen ion concentration, protein, oil and moisture contents and free fatty acid value before and after storage. The insects observed were *Dermestes maculatus* and *Lardoglyphus sp.*, the presence of these insect pests signified post-harvest and economic losses. Microorganisms identified were *Klebsiella pneumoniae*, *Pullularia pullulans*, *Fusarium sp*, *Microsporium gypseum*, *Scopulariopsis brevicaulis*, *Rhizopus japonicus*, and *Claudosporium herbarum*. Hydrogen ion concentration was between (3.60 - 5.37 and 3.60 - 5.39); Protein content was between (51 - 68% and 51-66%); moisture was between (14-24% and 15 - 26%); oil content was (5 - 32% and 3 - 18%); acid value ranged between 11-18 mgKOH/g and 37-86 mgKOH/g before and after storage, respectively.

Key words: Bio-deteriorating agent, smoked fish, temperature, insect pest activity, *Scopulariopsis brevicaulis*.

INTRODUCTION

Fish is the cheapest form of animal protein, vitamins, oils and minerals. It is a major living aquatic resource yet a perishable biomaterial especially in the tropics. It is a healthier meat option due to the high content of long chain polyunsaturated fatty acids (LCPUFA_s) which are associated with improving health and preventing diseases of old age (Kabaherda et al., 2009). Fish and fish products play a significant role in the diets of the populations of West African Countries and constitute more than 60% of the total protein intake in the rural areas. Fish is eaten fresh, preserved or processed (smoked) and form a much-cherished delicacy that cuts across socio-economics, age, religious and educational barriers in Nigeria (Adebayo-Tayo et al., 2008). It has an advantage over pork or beef as cheap source of protein because it has little or no religious rejection and provides

a significant portion of protein intake in the diets of a large proportion of the people, particularly in the developing countries.

Fish spoilage is accelerated immediately after catch because of the presence of high temperature and humidity in the tropics. Other factors include the poikilothermic nature of the fish, a high postmortem pH in the flesh, presence of non-protein nitrogen (NPN) in large quantities and the presence of trimethylamine oxide (TMAO) (Anonymous, 2009).

Poor handling, inadequate processing facilities, lack of ice or storage facilities, remoteness of fishing villages to urban market center and poor distribution channels as factors that contributed to reduction in fish utilization in the tropics (Ames, 1991).

Smoking has been described as the most commonly

used methods of fish preservation in the tropics when it is meant for storage. It is also noted that apart from giving the product a desirable taste and odour, it also provides a longer shelf life through antibacterial and oxidative effects, lowering pH, imparting colouration as well as accelerating the drying process and acting as antagonist to spoilage agents (Horner, 1997, Sengor et al., 2004; Olorokun et al., 2007; Abolagba and Melle, 2008). Furthermore, traditional fish preservation by using hard wood in preference over soft wood for smoking yields more bacteriologically stable products (Davies, 2006).

The effects of physical damage are manifested as weight loss and loss of visual quality which results in poor economy since intact (whole) smoked fish attracts higher price in market than fragmented once. Microbial action has been known to play a large part in the spoilage of fish (Eyo, 2001).

The objectives of the study were to examine physical quality of smoked fish samples, identifying insect pests associated with deterioration during storage to examine microbial quality of smoked fish samples by identifying microorganisms associated with deterioration during storage.

These objectives were investigated with a view to inform the public of the inherent danger in consuming the product.

MATERIALS AND METHODS

Samples collection

Five species totaling fifty (50) smoked fish samples were obtained from a local processing plant in Osogbo, Osun State. They were identified as *Clarias gariepinus* (10), *Hydrocynus spp* (10), *Parachanna obscura* (10), *Malapterurus electricus* (10) and *Clarias scopoli* (10). They were stored in different sterile bags at room temperature in the laboratory for a period of four months and were analyzed for physical quality, nutritional content and microbial load before and after storage.

Collection and identification of insect pests

Insect pests were collected separately from each sterile bag using a soft brush. The insects were examined under the microscope and were kept in preservative fluid (70% ethyl alcohol). Identification keys used were FAO (1989) and Odeyemi and Daramola (2000).

Moisture content determination

Five gram (5 g) of ground samples was dried to a constant weight in a mechanical convection oven with lids removed at 105°C for 4½ h. The samples were covered with lid and transferred to a desiccator at room temperature to cool and was weighed, lost in moisture content was calculated (AOAC, 1995).

Protein content determination

Digestion

0.5 g of well ground sample was weighed; a little scoop of digestion

catalyst was added and 20 ml of conc. sulphuric acid was also added slowly. After the reaction had subsided, the digestion flask was placed on a digester and it was digested for 2 h at 80°C until it became clear. It was then made up to 50 ml mark after cooling in a measuring cylinder.

Distillation

Fifty milliliter (50 ml) of boric acid was measured (plus indicator) into a 200 ml conical flask. The flask was placed under the receiving tube of the distillation unit in a way that the end of the tube is below boric acid level. Twenty milliliter (20 ml) of the sample was measured into Kjeldahl digestion flask and 50 ml of 40% NaOH was added carefully without shaking the flask.

The distillate was titrated with standard hydrochloric acid until the end point is reached that is when there is a clear fluid colour change.

$$\% \text{ Nitrogen} = \frac{\text{Titre value} \times \text{Acid concentration} \times 0.014 \times \text{Dilution factor} \times 100}{\text{Weight of sample}}$$

$$\% \text{ Crude Protein} = \% \text{ Nitrogen} \times 6.25$$

Oil content determination

Forty gram (40 g) of ground sample was folded in a piece of Whatman No. 2 filter paper and oil was extracted with Soxhlet apparatus using ethanol as the solvent without interruption for 6 h. It was allowed to cool and was poured into a known weight screw cap bottle. The ethanol was evaporated on a water bath until no ethanol was left and the constant weight was recorded.

The difference between empty weighed bottles and oil containing bottles gave the oil content.

Acid value determination

One gram (1g) of oil was dissolved in 50 ml of neutral solvent in a 250 ml conical flask. A few drops of phenolphthalein was added and titrated against 0.1N potassium hydroxide. It was shaken constantly until a pink colour which persists for fifteen seconds was obtained.

$$\text{Acid Value (mg KOH/g)} = \frac{\text{Titre Value} \times \text{Normality KOH} \times 56.1}{\text{Weight of the Sample (g)}}$$

Hydrogen ion determination

Two gram (2 g) of each blended samples was weighed in triplicates and made into fish slurry using 100 ml distilled water. The homogenate was allowed to stay for 5 min before taking readings using digital thermo pH meter and the average mean was calculated (AOAC, 1995).

Isolation of bacteria and fungi

Culture media preparation

Nutrient agar (NA) was used for the culture of bacteria and potato dextrose agar (PDA) was used for fungi. Twenty-eight gram (28 g) of NA and 37 g of PDA were dispensed differently in 1 litre of distilled water in separate conical flask. Sterilization was done in the

Table 1. Insect pests observed in different smoked-dried fish samples after storage (4 months) at room temperature.

Sample	<i>Dermestes maculatus</i> Beetle	<i>Lardoglyphus</i> sp.
<i>Malapterurus electricus</i>	-	-
<i>Hydrocynus</i> sp	-	+
<i>Clarias gariepinus</i>	+	+
<i>Parachanna obscura</i>	-	-
<i>Clarias scopoli</i>	+	+

-, Absent; +, present.

Table 2. Biochemical analysis of various smoked fish samples before and after storage for 4 months at room temperature.

Smoked fish samples	Protein content (%)		Oil content (%)		Moisture content (%)		Acidity value (mgKOH/g)		pH
	Before	After	Before	After	Before	After	Before	After	
	<i>Malapterurus electricus</i>	57	55	32	18	14	15	18	
<i>Hydrocynus</i> sp	60	55	12	10	21	26	13	37	3.63
<i>Clarias gariepinus</i>	68	66	28	16	22	23	15	53	5.35
<i>Parachanna obscura</i>	61	60	5	03	19	20	11	86	5.28
<i>Clarias scopoli</i>	51	51	19	16	24	26	13	50	3.60

autoclave at 121°C for 15 min after which it was allowed to cool. The culture media was shaken vigorously before use (Taylor et al., 1964).

Isolation

For each fish sample, five test tubes were used for serial dilution. One gram (1 g) of the blended samples was weighed aseptically and mixed thoroughly in 9 ml distilled water. Subsequent tenfold dilution was made up to 10^{-5} from it. One milliliter (1 ml) of each dilution was dispensed in sterile Petri dishes using pour plate method. The plates were allowed to solidify and were incubated at 35°C for 24 h (bacteria) and fungi were left on the bench for 3 to 5 days at room temperature. Bacteria and fungi count were done after 24 and 72 h, respectively.

All observed colonies were subcultured to obtain pure cultures which were subsequently isolated and identified at Microbiology Department, Obafemi Awolowo University Ile – Ife, Nigeria.

Statistical analysis

Duncan multiple range test (Duncan, 1955) was used to compare significant differences between the means in microbial population before and after storage while simple percentage and frequency was used to determine insect population.

RESULTS

After the period of four months, the study showed the presence of insect pests which was absent before storage. Table 1 shows *Dermestes maculatus* and *Lardoglyphus* spp. as types of insects' pest observed in

fish samples. Table 2 shows the biochemical analysis of various smoked fish samples before and after storage, respectively.

Microbial analysis

Tables 3 and 4 show the means of fungal and bacteria colony counts per smoked fish samples ($\text{cfu/g} \times 10^3$) before and after storage, respectively. Table 5 reveals the frequencies of occurrence of fungi isolated per smoked fish samples and fungi isolate respectively. Rate of occurrence (%) of fungi isolated per smoked fish samples is contained in Table 5.

DISCUSSION

Insect pest infestation

Insect infestation is controlled by include moisture content, water availability, physical structures and nutrient availability and by store temperature and relative humidity.

The insect pests that infested smoked fish samples were *D. maculatus* and *Lardoglyphus* sp., they were however absent in both *P. obscura* and *M. electricus*. It was also observed that different genera of smoked fish samples varied in their susceptibility to infestation by beetles and mites. This might be due to the physical structures, moisture content, nutritional composition,

Table 3. Mean fungal colony counts per smoked fish samples (cfu/g x 10³) for 4 months at room temperature.

Smoked fish samples	Before storage	After storage
<i>Malapterurus electricus</i>	0.0 ± 0.00 ^c	0.0 ± 0.00 ^d
<i>Hydrocynus sp</i>	2.5 ± 0.12 ^a	3.1 ± 0.13 ^a
<i>Clarias gariepinus</i>	1.6 ± 0.09 ^b	2.0 ± 0.03 ^b
<i>Parachanna obscura</i>	1.6 ± 0.06 ^b	1.9 ± 0.03 ^b
<i>Clarias scopoli</i>	1.5 ± 0.25 ^b	1.7 ± 0.09 ^c

Means with the same superscript along the column are not significantly different at P > 0.05.

Table 4. Mean Bacterial colony counts per smoked fish samples (cfu/g x 10³) for 4 months at room temperature.

Smoked fish samples	Before storage	After storage
<i>Malapterurus electricus</i>	1.4 ± 0.12 ^{ab}	1.8 ± 0.09 ^a
<i>Hydrocynus sp</i>	1.1 ± 0.09 ^b	1.4 ± 0.20 ^a
<i>Clarias gariepinus</i>	1.3 ± 0.09 ^b	1.8 ± 0.06 ^a
<i>Parachanna obscura</i>	1.2 ± 0.09 ^b	1.7 ± 0.19 ^a
<i>Clarias scopoli</i>	1.7 ± 0.12 ^a	1.9 ± 0.17 ^a

Means with the same superscript along the column are not significantly different at P > 0.05.

Table 5. Frequency of occurrence of fungi isolated per sample for 4months at room temperature.

Fungi isolates	A	B	C	D	E
<i>Pullularia pullularis</i>	--	--	--	--	+
<i>Rhizopus japonicus</i>	--	--	--	+	+
<i>Cladosporium herbarum</i>	--	+	--	--	--
<i>Microsporium gypseum</i>	--	+	--	--	--
<i>Scopulariopsis brevicaulis</i>	--	--	--	+	--
<i>Fusarium sp</i>	--	+	+	--	--
Total	-	3	1	2	2
% Occurrence	-	38	13	25	25

-- absent, + present, A = *Malapterurus electricus*, B = *Hydrocynus* species, C = *Clarias gariepinus*, D = *Parachanna obscura*, E = *Clarias scopoli*.

possible deterrent, of the smoked fish samples. This is in line with the findings of Akintola and Lawal (2011) and FAO (1989); they both reported nutritional composition of some smoked fresh water fish as factors of resistance to insect attack.

The genus *Clarias* was observed to be more susceptible to *D. maculatus*, this may be due to its low salt tolerance since fish of fresh water origin are more frequently attacked by beetles. The genus *Hydrocynus* was observed to be infested by mites only. This might be an indication of re-absorption of moisture from the store environment which led to high occurrence of fungi on the smoked fish samples since mites are fungi feeders.

Effects of insect pests include loss of weight due to feeding damage, quantitative loss of smaller fragments, loss in visual quality, poor economy, reduction in

nutritional value and provision of conditions suitable for mould growth on fish. Significant differences were recorded at P < 0.05 in the microbial counts after storage.

The fungi species observed from all fish samples were dematiaceous moulds that are widely distributed in indoor environment, wood, soil, textiles, plant debris, and rotten organic material, they are frequently isolated as contaminants on foods. Fish treated this way is prone to contamination with microorganisms such as bacteria and fungi. The occurrence of *Fusarium* species might lead to food poisoning through production of mycotoxins. The occurrence of *Rhizopus japonicus* might be due to re-absorption of moisture that supports its growth from storage environment (Christannah and Fagade, 2010). *Cladosporium* sp has been reported to produce epicladosporic and fagicladosporic acid (Joffe, 1965),

mouldiness of smoked fish should be viewed with various concerns because of the ability of the moulds to produce mycotoxins some of which are very dangerous and lethal to human and animals even in small doses.

The pathogens isolated and identified from smoked fish, which were not normal flora, are of food and public health implication. They are not normal flora of the fish.

pH and moisture content

Microbial growth and spoilage of food is affected by both pH and moisture content. Higher moisture content is conducive to fungi infestation and moisture loving insects thus food becomes rapidly deteriorated. The acceptable moisture content of smoked fish samples is 14.0% (FAO, 1989). Nearly all the samples have their moisture content to be higher than 14.0% before and after storage except *M. electricus* that had 14% and 15% before and after storage respectively. The increase in moisture contents observed in smoked fish samples (as shown in Table 2 could be due to re-absorption of moisture from the environment or improper drying of the fish). This provides an enabling environment for both moisture loving insects and microorganisms. Effects of moisture damage include quality loss and fungi development which led to the formation of mycotoxins.

The pH of living fish is generally between 6.7 and 7.0, and is subjected to variation with time of the year, feeding, and degree of activity of the fish (Woyewoda et al., 1986). After death, glycogen stored in the muscle is broken down by glycolysis to produce lactic acid which in turn lowers the pH. Doe (1998) reported that pH in fish tissues generally drops to 6.5 or less after smoking. The pH observed in the smoked fish samples were acidic (as shown in Tables 2 and 3) and may also serve as important attraction for fungi. The highest fungal count was observed in *Hydrocynus* sp at 3.1×10^2 at a pH of 3.65 after storage. The microbial levels obtained from this result could be considered hazardous to consumers because of the possibility of enterotoxigenic strains. The bacteria count ranged between 1.4×10^3 and 1.9×10^3 and the highest bacterial count was observed at 1.9×10^3 in *Clarias scopoli* after storage.

Protein content in smoked fish increases due to an increase in the dry matter content per unit weight following sample dehydration during smoking (Goktepe and Moody, 1998) but in this work storage time did not really affect the protein content of the smoked fish samples. This finding is in line with the report of Ligia (2002) but contrary to the findings of Christiannah and Fagade (2010) that reported degradation of the proteins of smoked fish through proteases production by fungal isolates with *A. ochraceous* having the highest rate of degradation followed by *A. niger*, *Rhizopus* sp and *A. flavus*.

Acid value serves as a reliable indicator for fungi activity and of flavours in stored food commodity. It is

unlikely that foods with high acid value are unpalatable but there is danger of poisoning through mycotoxins generated by fungi if affected food is consumed either by animals or man. *Parachanna obscura* was observed to have the highest acid value after storage (Table 2) and this might be due to enzymatic breakdown of fat to free fatty acids during storage.

Malapterurus electricus had no incidence of insect pests and fungi occurrence but had acceptable moisture content according to FAO (1989). The absence of both fungi and insect pests might be due to good processing and storage. *Hydrocyanus* spp has the highest rate of fungi occurrence (that is 37.5%). High moisture content and nutrient availability in smoked fish samples may contribute to high rate of fungi occurrence. These fungi serves as food for mites which was the only observed insect pests on *Hydrocynus* spp and thus affected the acid value because of the reduced mould activity.

The study reveals the insect pests that are of economic importance and microorganisms that are of veterinary, medical and public health implications.

Bacterial infection of fish and fish products may influence human health by inducing disease/infection and cause abdominal pain, acute gastroenteritis, bloody/mucoid diarrhoea, nausea, vomiting and fever upon ingestion of insufficiently heat-treated (smoked) fish or fish products contaminated during the processing and the presence of these bacteria harmful to man generally indicates poor sanitation in handling and processing (Kam et al., 1995; Han et al., 2001).

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