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EFFECT OF TRADITIONAL PRESERVATION METHODS ON THE MICROBIAL AND NUTRITIONAL CHARACTERISTICS OF FISH (*Scomber scombrus*)

Oshoma*, C. E. and Eseose, H.

ABSTRACT

*Department of Microbiology, Faculty of Life Sciences, University of Benin, P.M.B. 1154 Nigeria. *Correspondence author E-mail: cyprian.oshoma@uniben.edu, +2349099727664.*

Microbial and nutritional composition of fish (Scomber scombrus) using traditional methods of preservation was assessed and compared with refrigeration preservation method, during storage. Methods of fish preservation used were drying, smoking, salting and refrigeration as control. The traditionally preserved fish samples were analysed for microbial and nutritional composition for duration of 4 weeks. During storage of fish samples, the highest bacterial load of $1.94\pm0.04\times10^5$ cfu g^{-1} was recorded for salted preservation method after 4 weeks while the least was $4.70\pm0.28\times10^3$ cfu g^{-1} for the control. The highest fungal count was $2.02\pm0.03\times10^4$ cfu g^{-1} while the least $4.80\pm0.14\times10^2$ cfu g^{-1} for salted and control preservation methods respectively after 4 weeks of storage. The bacterial isolates identified were Bacillus subtllis, Streptococcus pyogenes, Staphyococcus aureus, Corynebacterium spp., Enterobacter aerogenes, Serratia marcescens, Staphylococcus epidermidis and Pseudomonas aeruginosa. The fungal isolates identified were Mucor piriformis, Rhizopus stolonifer, Aspergillus niger, Penicillium expansuum and, Saccharomyces cerevisiae. Proximate composition of fish after 4 weeks of storage showed that the highest moisture content 65.30±3.64 and crude protein 48.61±2.21% were from the control and smoked fish respectively. It was observed that mineral compositions such Ca, Mg, Zn, Cu and Mn were found to decrease in value after 4 weeks of storage for the different traditional methods of preservation. The investigation indicated that traditional methods of fish preservation mostly smoking could be useful to preserve the nutritive values of fishes and possibly reduce post-harvest losses.

Keywords*: Scomber scombrus, fish preservation spoilage, microbial load.*

INTRODUCTION

Fish a highly perishable food item (Agbon *et al*., 2002), soon after death, are known to bio-deteriorate almost immediately. In the healthy live fish, all the complex biochemical reactions are balanced and the fish flesh is sterile (Tawari and Abowei, 2011). After death, irreversible change that results in fish spoilage begins to occur (Akinola *et al*., 2006). A variety of factors is accountable for fish spoilage. The quality of capture such as the fish health status, the presence of parasites, bruises and wounds on the skin and the mode by which the fish was captured are important at determining the rate of spoilage. The handling and the preservation practice employed after capture also affects the degree of spoilage of the fish (Akinneye *et al*., 2007). Fish preservation is the process whereby fish products are kept in its original state or an enhanced state to prevent microbial spoilage (Adeosun *et al*., 2015). Fish is highly susceptible to deterioration without any preservative or processing measures (Latifa *et al*., 2014; Olaleye and Abegunde, 2015). Latifa *et al.* (2014) reported that immediately the fish dies, a number of physiological and microbial deterioration set in and thereby degrade the fish. Pathogenic microbes isolated from different species of fish were able to grow and produce toxic metabolites and are retained in fish even after processed and stored. These toxic metabolites can be a serious public health hazard (Olaleye and Abegunde, 2015). Fish is a major source of protein and its harvesting, handling, processing and distribution provide livelihood for millions of people as well as providing foreign exchange earning to many countries (Al-Jufaili and Opara, 2006). Appropriate processing of fish enables maximal use of raw material and production of value-added products, which is obviously the basis of processing profitability (Tawari and Abowei, 2011).

Majority of the traditional methods used in fish preservation involve the removal of water (drying), addition of phenol and other organic components (smoking), and alteration in pH concentration (salting or brining) (Adeosun *et al.*, 2015). The method that may be employed determines the end product flavour and texture. In order to develop optimum preservation techniques for these value added products in active forms, understanding of the mechanism responsible for their degradation is essential. Fresh fish spoilage can be very rapid after is been harvested from the water body. The spoilage process (Rigor mortis) will start within 12 hours of their catch in the high ambient temperatures of the tropics (Berkel *et al*., 2004). Rigor mortis is the process through which fish loses its flexibility due to stiffening of fish muscles after few hour of its death (Adebowale *et al*., 2008). During fish spoilage, there is a breakdown of various components and the formation of new compounds. These new compounds are responsible for the changes in odour, flavor and texture of the fish meat. This represents a major concern of the freshness of saleable products and the breakdown of proteins and lipids. Higher energy demanding freeze-storage preservation can be altered by synthetic or natural preservatives for control of lipid oxidation and microbial growth in fish during storage (Mahmoud *et al*., 2006). Combination of these preservatives and refrigeration diminishes the process of spoilage (Chakroborty and Chakroborty, 2017). The rate of deterioration

during the storage of a product depends on the biochemical compositions of substrates and metabolites in the tissue, the microbial contamination, and the condition of storage. Therefore, this study aimed to assess the microbial and nutritional composition of fish (*Scomber scombrus*) using traditional methods of preservation.

MATERIALS AND METHODS

Sample collection and preparation for analysis

In this study, twenty (20) fish (*Scomber scombrus*) samples of an average length to width ratio of 20:5 were purchased from the cold storage room at New-Benin Market, Benin City, Nigeria. The samples were rinsed with sterile distilled water. The fish samples, five (5) each, of approximately same size and weight were subjected to different methods of preservations such as smoking, salting and drying respectively. The last five (5) fish samples were untreated but stored in the refrigerator. The fish samples were smoked using traditional method of smoking according to the modified method of Sani *et al*. (2016) using a smoke house drum. The fish samples were laid on iron spits and fire of hardwood was made at the bottom of the smoke house drum. During preparation, the fish samples were weighed to determine their initial moisture content prior to smoking. The prepared samples were then placed over the spits. During the smoking operations the top of the drum was well covered with a sack; the fire control opening was also closed. The smoking was completed after six days.

Salting treatment was carried out by completely immersing the fish into Sodium Chloride solution of 2.4% (w/v) for 20 min according to the method of El-Bassir *et al*. (2015). A traditional oven with charcoal from burnt wood materials as the source of energy was prepared and the charcoal lit to heat up the oven. The prepared fish samples were arranged inside the oven and the doors were shut tight. They were turned over every 2 hours so as to dry quickly and to prevent deterioration. This process was closely monitored for 48 hours with intermittent turning to prevent the sample from getting burnt. Upon completion of the drying period, the already dried samples were allowed to cool at room temperature. All the treated fish samples with different preservatives were kept accordingly in a dry clean plastic container sterilized with 70% ethanol solution and stored in a box where they are protected from dust and insects. Also, untreated fish samples were refrigerated at temperature 0 - 4.0 °C, which served as the control. Microbial load, proximate analysis and mineral content of fish were analysed every week for duration of 4 weeks.

Microbial enumeration of fish sample

Microbial enumeration was carried out according to the procedure of Cheesbrough (2000). A 10 g of each of the fish samples was cut from the mid regions with a sterile knife, ground aseptically in porcelain mortal and mixed with 90 ml of sterile distilled water. From this mixture 10-fold serial dilution was carried out in cleaned sterile test tubes containing 9 ml of sterile distilled water. From the aliquots, 0.1 ml was transferred into Nutrient agar (NA) and 0.1 ml was transferred into Potato dextrose agar (PDA) plates. Plates containing NA were incubated at 37 °C for 48 hours and colonies counted while PDA plates were incubated at 28±2 °C for 72 hours. The discrete colonies on the Nutrient agar and Potato dextrose agar were selected and counted. The mean colony count on the nutrient agar and potato dextrose plates of each given dilution was used to estimate the total viable count for the samples in colony forming units per gram (cfu g^{-1}).

Identification of microbial isolates

From the Nutrient agar (NA) plates, colonies were randomly picked and repeatedly sub-cultured on Nutrient agar (NA) for purification. Purified bacterial isolates were stored in Nutrient agar (NA) slants for further studies. The purified bacterial isolates were characterized by morphology, Gram's reaction and biochemical test using the scheme in Bergey's manual of determinative bacteriology (Holt *et al*., 1989; Cheesbrough, 2000).

Fungal isolates were examined based on cultural and morphological characteristics. Lactophenol cotton blue staining (needle mounts technique) was employed and examined under the microscope at 40X objective. Their identification was performed according to the procedure of Barnett and Hunter (1972) and Larone (1986).

Determination of proximate composition

Ash content, crude fibre, crude protein, moisture content, carbohydrate and fat content were determined using standard procedures prescribed by AOAC (2003).

Determination of mineral composition

Sodium, Potassium, Calcium, Magnesium, Copper, Iron, Manganese, Zinc, Phosphorus, Selenium were determined by transferring 10 g of fish sample into a 250 ml conical flask containing 100 ml of 0.1M HCI, stoppered, and then shaken for 30 minutes. The mixture was filtered through Whitman filter paper No.42 and then Metals (and some inorganics) were determined in the filtrate by Atomic Absorption Spectrometer following the procedures recommended by AOAC (2003).

Statistical analysis

All assays were carried out in duplicates, and the means and standard deviations (SD) were determined. The effect of methods after preservation was evaluated by comparison of the microbial characteristics of the processed fish samples at the end of the storage period (week 4) using t test. All statistical analysis were carried out using SPSS version 23.

RESULTS

Changes in bacterial load of dried, smoked, salted and refrigerated (control) fish samples stored for a period of 4 weeks is shown on Figure 1. The highest viable bacterial load 1.94±4.00× 10^5 cfu g^{-1} was observed in the salting method of preservation after 4 weeks of storage while the least 4.70±0.28 × 10^3 cfu g^{-1} was from the control. Statistically, there was significant difference in the bacterial load of samples obtained from the various preservative methods employed ($p<0.02$), after 4 weeks of storage. Changes in the fungal load from the various preservation methods are shown in Fig. 2. Fungal growth was observed to be highest (2.02±0.30× 10^4 cfu g^{-1}) after 4 week of storage from salting preservation method while the lowest (4.80±0.14× 10^2 cfu g^{-1}) was from the control. In comparing the fungal load from various method showed statistically significant difference ($p<0.01$).

The bacterial isolates identified from the various preservation methods were found to be *Bacillus subtllis, Staphylococcus epidermidis, Streptococcus pyogenes, Staphyococcus aureus, Corynebacterium* sp., *Enterobacter aerogenes, Pseudomonas aeruginosa* and *Serratia marcescens* as shown in Table 1. The highest in the frequency of occurrence among the isolates was *Staphyococcus aureus* (19.1%) while *Enterobacter aerogenes* had the lowest percentage occurrence of 1.0%. The identified fungal isolates were *Mucor piriformis, Rhizopus stolonifer, Aspergillus niger, Penicillium expansum* and *Saccharomyces cerevisiae* (Table 2). Of the fungal isolates, *Aspergillus niger* was the most prevalent isolate occurring in the fish samples from the different preservative methods with a percentage frequency of 29.0% while *Saccharomyces cerevisiae* was the least isolated fungi with a percentage frequency of 7.3%.

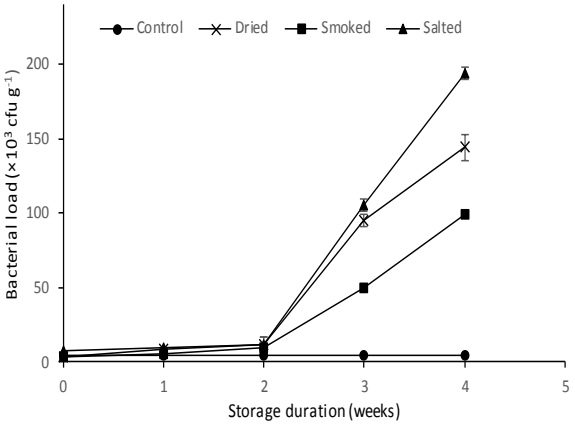

Fig. 1: Bacterial load (cfu g^{-1}) of traditionally preserved fish samples during storage

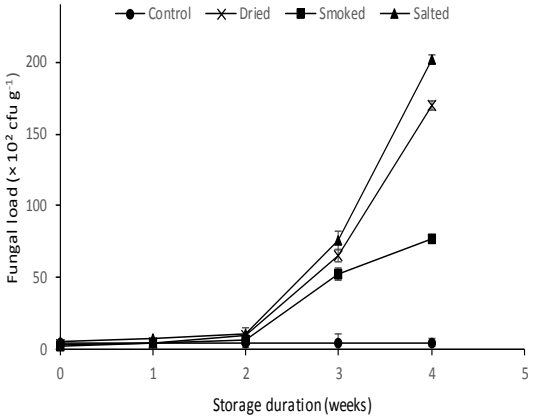

Fig 2: Fungal load (cfu g^{-1}) of traditionally preserved fish samples during storage.

Table 1: Occurrence of bacterial isolates on traditionally preserved fish samples

Bacterial isolates	Occurrence (%)
Bacillus subtllis	18.1
Staphylococcus epidermidis	13.3
Streptococcus pyogenes	18.1
Staphyococcus aureus	19.1
Corynebacterium sp.	19.1
Enterobacter aerogenes	1.0
Pseudomonas aeruginosa	9.5
Serratia marcescens	1.9

Table 2: Occurrence of fungal isolates on traditionally preserved fish samples

Fungal isolates	Occurrence (%)
Mucor piriformis	26.1
Rhizopus stolonifer	13.0
Aspergillus niger	29.0
Penicillium expansum	24.6
Saccharomyces cerevisiae	7.3

Proximate analysis of fish samples subjected to different traditional methods of preservation during storage is presented in Table 3. Moisture content was observed to be highest (65.39±3.64%) in control sample while smoked fish recorded the lowest (14.54±0.98%) after 4 weeks of storage. Crude protein was high in the smoked fish (48.61±2.21) after 4 weeks of storage than dried (45.82±0.57) and salted (10.54±1.21) fish. Salted fish recorded the highest carbohydrate content (13.81±1.39%) after 4 weeks of storage. Mineral composition of fish samples preserved traditionally after four weeks of storage is presented in Table 4. In all the different traditional methods of fish preservation, mineral compositions such Ca, Mg, Zn, Cu and Mn were found to decrease in value after 4 weeks of storage. However, smoked fish after 4 weeks of storage recorded the highest values of 2907.02±23.29, 981.77±3.44 and 1190.24±25.32 mg kg^{-1} for calcium, magnesium and iron respectively. The lowest calcium content (2357.36±105.78 mg kg^{-1}) was observed in samples preserved traditionally by drying. Sodium content had the highest value (946.81±6.37 mg kg^{-1}) in the salted fish sample at week 4 while the least (743.06±11.37 mg kg^{-1}) was from control fish sample.

DISCUSSIONS

Fish, due to their soft tissues and good source of nutrients, such as protein, vitamins and minerals, are prone to microbial contamination during storage (Al-Jufaili and Opara, 2006; Abolagba *et al.*, 2011). Fish handlers could engender microbial contamination due to unhygienic methods of traditional processes (Sani *et al.*, 2016). From the total samples of traditionally preserved fish examined, all revealed steady increase of bacterial load over a period of 4 weeks. These results agree with the report of Adeosun *et al*. (2015); Olaleye and Abegunde (2015; Chakroborty and Chakroborty (2017) that bacteria are primary contaminants of smoked fish. However, Akise *et al*. (2013) reported that the quality of fish is rarely determined by the microbial load but an indication that spoilage can be induced by microorganisms, which may likely affect the health condition of consumers. From microbiological point of view, microbial load greater than 10^6 cfu g^{-1} is generally unacceptable and unfit for human consumption (Cheesbrough, 2000; Chakroborty and Chakroborty, 2017). The highest bacterial load in the salted fish after 4 weeks of storage is less than the maximum permissible limit. However, there is an indication that these bacteria can continue to proliferate if allowed for longer storage duration. The continuous increase in bacterial growth could occur as a result of fish being a good source of growth medium for the organisms and the level could be considered as potentially hazardous, with these levels (10^4 cfu g^{-1}) of contamination, which may result in food-borne illness if consumed (Moshood and TengkuHaziyamin, 2012). .

Table 3: Proximate analysis of Fish samples preserved using various traditional methods after 4 weeks of storage

Proximate analysis (%)	Control		Dried		Smoked		Salted	
	0	4	0	4	0	4	0	4
Moisture	66.67±4.19	65.30±3.64	25.3±1.13	16.00±2.77	21.44±1.78	14.54±0.98	62.4±3.56	50.27±2.00
Crude Protein	16.44±1.08	16.18±0.88	38.15±0.42	45.82±0.57	39.21±1.29	48.61±2.21	18.32±1.63	10.54±1.21
Crude Fat	9.34±0.16	9.00±0.13	22.01±0.98	21.11±1.30	19.79±0.47	18.04±0.06	10.74±2.07	7.47±0.64
Crude Fibre	1.00±0.03	1.07±0.18	5.90±0.59	3.30±0.39	6.15±0.67	4.56±0.64	1.46±0.20	4.85±0.42
Ash Content	1.89±0.05	1.88±0.04	6.28±0.11	6.29±0.98	6.19±0.24	7.16±0.09	2.53±0.18	8.84±0.34
Carbohydrate	0.86±0.06	0.76±0.09	4.22±0.26	4.02±0.05	5.45±0.60	5.26±0.09	2.13±0.06	13.81±1.39

Table 4: Mineral concentrations (mg kg^{-1}) of fish samples preserved using various traditional methods after 4 weeks of storage

Mineral composition (mg kg^{-1})	Control		Dried		Smoked		Salted	
	0	4	0	4	0	4	0	4
Calcium	2764.5±63.78	2815.07±11.82	3823.83±33.73	2357.36±105.78	4495.06±8.63	2907.02±23.29	3194.43±36.93	2258.06±19.20
Magnesium	927.125±56.53	969.7±3.56	995.53±13.76	926.23±8.61	1221.72±29.13	981.77±3.44	1196.07±18.15	763.97±6.32
Potassium	1409.9±3.82	1429.15±36.81	1297.8±3.25	7364.21±25.95	1310.84±16.77	1932.20±11.82	1402.55±13.36	2239.75±6.70
Sodium	734.1±0	743.06±11.24	881.15±30.45	927.07±9.01	880.68±12.19	946.81±6.37	1188.1±25.31	923.45±11.24
Phosphates	838.915±19.31	834.06±11.37	837.82±4.03	1246.26±8.60	809.62±10.44	1610.57±3.08	804.53±8.32	1434.85±14.84
Iron	89.515±3.49	93.31±2.69	128.21±8.22	621.79±15.87	71.38±0.89	1190.24±25.32	83.41±0.88	179.49±9.31
Zinc	11.09±0.58	11.98±0.56	20.9±1.56	18.33±0.39	26.19±1.43	17.31±1.00	10.93±0.10	9.91±0.15
Copper	21.6±0.00	22.36±0.74	26.4±0.85	13.13±0.04	16.80±0.85	17.34±0.09	16.82±0.83	26.11±0.43
Manganese	7.91±0.16	7.75±0.11	20.005±1.42	13.36±0.06	14.20±1.13	13.77±0.04	13.75±0.49	10.99±0.83
Selenium	0.21±0.04	0.19±0.00	0.155±0.02	0.19±0.00	0.19±0.03	0.20±0.05	0.18±0.01	0.14±0.06

The difference in the mean bacteria counts of samples obtained from the three different traditional preservative methods employed showed that the quality standards of the product varied. This was due to the differences in the method of processing, storage and handling of the fish samples. Samples preserved by salting had the highest degree of bacterial contamination all through the duration of the study.

Normal microbial flora of fish are often beneficial as they safe-guard against invasion of flesh by other microbes, however, they graduate to becoming pathogenic when enabling environment (temperature and relative humidity) in association with bad handling, poor hygiene, and delayed processing and preservation favours population growth (Abolagba *et al.*, 2011). The bacterial isolates such as *Staphylococcus aureus*, *Bacillus subtllis* and *Pseudomonas aeruginosa* had been reported as normal flora of fish (Abolagba *et al.*, 2011; Al-Reza *et al.*, 2015). The spoilage potentials of *Staphylococcus aureus* on fish during storage have been highlighted in the report of Al-Reza *et al.* (2015). The presence of these organisms in the preserved fish samples might be due to high moisture content level of the product during storage and storage in temperature, which favours the growth of these organisms thereby resulting to fish spoilage. During handling of fish, the natural flora of the fish environment will be contaminated with organisms associated with man, such as members of the Enterobacteriaceae and *Staphylococcus aureus* (Abolagba *et al.*, 2011).

The investigation also revealed that all the traditional methods of preservation allowed fungi to proliferate in the fish samples. Among the fungal isolates, *Aspergillus*, *Mucor* and *Penicillium* were the dominant species identified from the various preservation method used and corroborate Sani *et al.* (2016) report. The presence of these fungal isolates, mostly *Aspergillus* and *Penicillium* in the fish product not only downgrade the quality but might result to toxic metabolites production like mycotoxins (Al-Reza *et al.*, 2015). In addition, the unacceptable direct contact

with the hands during processing (Mepba *et al.,* 2008) could be an alternative source contributing significantly to the prevalence of different microbes.

The moisture content is a pointer to the rate at which deterioration occurs in fish samples resulting in the early decomposition. The moisture content of smoked fish and dried fish was lower than that of salted fish. Adeosun *et al*. (2015) reported that fish spoilage as a result of bacteria action and enzyme activities can be reduced by reducing moisture content of fish hence, smoking of fish can be adjudge to be an ideal preservative method (Ghaly *et al*., 2010). The high crude protein content in the smoked fish than other methods infers that loss of protein nitrogen was not high during smoking of fish. Probable reason is that crude protein component of fish is been concentrated through smoking method (Al-Reza *et al*., 2015). The results of the proximate compositions in this study were slightly different from those of Adebowale *et al.* (2008) who reported the range of moisture, protein, fat and ash content of Nigerian smoked catfish to be 7.16-10.71, 33.66-66.04, 1.58-6.09 and 9.21-12.16%, respectively. This could be due to differences in species of fish. Decrease in crude fibre value recorded in the dried and smoked fish samples could be due to loss of moisture content during storage.

This study revealed that traditionally preserved *Scomber scombrus* help in retaining macro and micro mineral elements, found in fish, in spite of the processing effects and may contribute to health, growth and development of human beings. The result of the mineral composition reduced with increased storage duration. Although there was a similar pattern of change in the mineral composition for the various preservation methods but, there seems to be variation among the methods. Variation in mineral composition of fish from the various preservation methods could be the effect of moisture content of fish after processing (Mphande and Chama 2015). The content of calcium, magnesium and iron were higher in smoked fish than other methods. Calcium and magnesium content observed in all the fish samples mostly the smoked fish is of nutritional benefits due to their need in bone formation (Aremu *et al.*, 2013). Hence, smoking of fish as a means of preservative should be encouraged. Mphande and Chama (2015) reported that microbial activities on the fish during storage impacted negatively on the mineral composition of fish hence, the low values in most parameters analysed after 4 weeks. This is also a confirmation on the variations recorded in the mineral compositions of fish.

CONCLUSION

The microbiological and nutritional compositions of the traditionally preserved fish (*Scomber scombrus*) showed variations from one preservative method employed to another. The results however indicate that smoking and drying methods are two very important preservation methods, which could preserve the nutritive values of fishes and possibly reduce post-harvest losses. However, good smoking and drying methods should be adopted, hygienic and proper storage devices put in place.

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