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BIOPHYSICOCHEMICAL CHANGES THAT OCCUR IN FISH DURING DIFFERENT STAGES OF TRADITIONAL PROCESSING

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ABSTRACT

Smoking in Nigeria is the most affordable and widely used traditional fish processing method aimed at preserving or reducing losses. However, smoked fish can be a source of microbial hazard. This study assessed the biophysicochemical changes of fish muscles along the processing line of *Oreochromis niloticus* in Eleyele lake. A Structured questionnaire was administered to the fish processors in order to identify the procedural processing line of their fish. Microbial assay was done for total aerobic bacteria, enterobacteriacea, and salmonella counts. Nine processing stages identified in fish processing include: Stage 1- unwashed fish muscle tissue; Stage II- washed fish muscle tissue (washed, scalded, eviscerated and washed again); Stage III- Salted fish at 28.9^{0}C; Stage IV- Salted Fish in the smoking kiln after 30 minutes at 75^{0}C (temperature of smoking flame); Stage V - Salted fish in the smoking kiln after 1hr at 76 ^{0}C; Stage VI- Salted fish in the smoking kiln after 1hr 30min. 65^{o}C; Stage VII- Salted fish in the smoking kiln after 2hr at 40^{0}C; Stage VIII -Salted fish in basket after 24hrs of storage; and Stage IX- Salted fish in basket after 48hrs of storage at 26-28^{o}C ambient temperature. A significant increase ($P<0.05$) was recorded in total aerobic plate count (TAPCs) from stage I (7.42CFU/g) to II (12.00CFU/g) when the tilapia fish was washed using the water from the lake, scalded and eviscerated. The reverse was the case with the enterobacteriaea counts (EC). At stage III, where salt was sprinkled on the fish, a significant decrease ($P<0.05$) was observed in the TAPCs from 12.00$\pm$0.00CFU/g to 9.17$\pm$3.22CFU/g. Also, a significant decrease ($P<0.05$) was observed in all the counts in the first 30 minutes of smoking (stage IV) when the temperature rose from 28.9^{0}C to 75^{0}C. Furthermore, a significant decrease ($P<0.05$) was recorded only in the TAPC while a significant increase ($P<0.05$) was observed in EC and Salmonella counts. A significant increase in TAPCs was observed during storage from 7.56 $\pm$0.10CFU/g (stage 7) to 12.0$\pm$0.00CFU/g (stage 8) while a significant increase ($P<0.05$) was observed in ECs from stage 8 to 9. Significant differences ($P<0.05$) were obtained for zinc, manganese, iron along the processing line, but Lead, Cadmium, Chromium, Nickel and Copper showed no significant differences. This study showed significant changes in the biological, physical and chemical changes of fish muscles along the processing line of Tilapia. The implications and public health concerns are hereby discussed.

Key words: Smoking, *Oreochromis niloticus*, processing, bacteria, metals

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INTRODUCTION

Smoking is one of the traditional fish processing methods aimed at preventing or reducing post harvest losses [1]. However, smoked fish and shellfish products can be a source of microbial hazards [2, 3]. Salmonella and *Escherichia coli* are the major causes of foodborne diseases from smoked tilapia. Many studies have been documented on Eleyele Lake but there is scarce information on the changes in microbial load, heavy metals or trace elements and nutritional contents at different stages of processing and storage of Tilapia. This study was aimed at determining the biophysical changes that occur in fish during different stages of traditional processing and storage.

MATERIALS AND METHODS

Lake Eleyele is located on latitude 7^0 25N and longitude 3^0 55E in the metropolitan city of Ibadan. It derives its source from river Ona. The lake was constructed in 1942 and is a length of 240m across the dam and catchments area of 323.7sq km. The lake is 125m above sea level with an average depth of 6.0m. The lake boasts of a number of fish species including the families Gymnachidae, Characidae, Cichlidae, Clariidae and Cyprinidae among others.

Experimental Procedure

Appropriate ethical approval was received before the commencement of this study**.** A structured questionnaire was administered to the fish processors near the Eleyele Lake. This was done to determine the processing stages and storage conditions for the fish.

Ten fish of weights 472$\pm$27.69g were used for this study. After the tilapia had been cropped, the fish were washed with water collected from the lake. This was followed by scaling, evisceration, washing to remove blood stains, salting and smoking. The fish were then stored at ambient temperature in a basket for 3 days as is the normal traditional method of processing and storage.

Fish muscle samples were taken in two replicates at each stage of processing. Samples during the smoking stage were taken at 30minutes interval. Sterilized knives were used in cutting 5g of the fish muscle at both sides. These samples were collected in sterile Stomacher bags, sealed and chilled in ice block packs to maintain the microbes and floral loads.

Water samples were collected for analysis of physicochemical parameters with the aid of sterile sampling bottles from the source, middle and edge of the lake thrice. The oxygen bottle was used to take water samples for dissolved O_2. Fifty milliliter water samples were collected from the source for water quality analysis. The middle and end of the lake were sampled and analysed for dissolved Oxygen, total hardness, pH and total dissolved solids using HACH freshwater aquaculture test kit (FF-1A).

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Temperature of the smoking flame at every point of processing (precisely 30 minutes interval) was taken with the aid of a thermometer (TBT-08H, China).

Fish processors store smoked fish for up to 3 days. It was on this premise that the time for the storage period in this work was based. Samples were collected at these nine (9) stages of processing: Stage 1- unwashed fish muscle tissue; Stage II- washed fish muscle tissue (washed, scalded, eviscerated and washed again); Stage III- Salted fish at 28.9^{0}C; Stage IV- Salted Fish in the smoking kiln after 30 minutes at 75^{0}C; Stage V - Salted fish in the smoking kiln after 1hr at 76 ^{0}C; Stage VI- Salted fish in the smoking kiln after 1hr 30min. 65^{o}C; Stage VII- Salted fish in the smoking kiln after 2hr at 40^{0}C; Stage VIII -Salted fish in basket after 24hrs of storage; Stage IX- Salted fish in basket after 48hrs of storage at 26-28^{o}C ambient temperature.

Microbial assay

To determine total aerobic bacteria, enterobacteriacea, and salmonella counts in samples, five gram fish samples were homogenized in 10mls sterile 0.1% peptone water in a sterile stomacher bag using a stomacher machine. Samples were then serially diluted in 0.1% peptone water to 10^4 dilution levels. An aliquot of 0.1ml of the final dilution of each sample was then surface plated on plate count agar, macConkey agar and deoxycholate citrate agar for enumeration of total aerobic bacteria, enterobacteriacea and salmonella counts, respectively. Plates were incubated at 37^{o}C for 24hrs. After incubation colonies were counted using a colony counter and expressed in Log_{10}CFU/g.

Proximate Analysis

The determination of the crude protein, moisture, ash and fat contents of the fish were carried out in triplicate in accordance with earlier methods[4].The protein content was obtained through the determination of total nitrogen by micro Kjeldahl's method [5]. The value of nitrogen obtained was then multiplied by 6.25 to get the crude protein value. The ash content was determined by heating the samples to a temperature of 550^{o}C. The residue from the heating process is equivalent to the ash content. The fat content was determined by extraction with hexane by soxhlet's method [5].

Heavy Metals and Trace Element Determination

Manganese, lead, cadmium, copper, iron, nickel and zinc were determined with the aid of Buck Model 200 Atomic Absorption Spectrophotometer (Buck Scientific, America)[8].

Of sampled fish muscle, 0.5g was weighed into a set of digestion tubes. Ten (10) ml of perchloric and nitric acid was digested into the sample vessels. Mixture of content of the digestion tubes were then digested at a temperature of 120^{o}C for 2 hours on the digestion block. The digestion was followed by cooling at room temperature. Thereafter, samples were mixed with ultra-pure water to make a volume of 50ml. The samples were then transferred to a set of centrifuge tubes and shaken for 10minutes. The shaken sample solutions were transferred to a centrifuge and centrifuged at the rate of 4500rpm for 5minutes. The supernatants were then decanted into a set of Pyrex

glass. The combined stock standard (Pb and Cd (1000ppm each) was prepared from reference standards and stored in the refrigerator until use. The method of calibration curve was used for calibration and quantification of the AAS to its effective position. The certified sensitivity check sample for each element was used to optimize the efficiency of the AAS getting 0.200 absorbance for each sensitivity check for standard solutions aspirated during the measurement for each element. The working standards were first determined to create the standard curve; this was followed by the measurement of the unknown analytes. The atomic absorption spectrophotometer was adjusted to specific wavelength corresponding to each of the metals to be measured.

Statistical Analysis

Statistical analysis was performed using SPSS 13.0 for Windows. Analysis of Variance (ANOVA) was used and statistical significance was set at $P<0.05$. Duncan Multiple Range Test was used to separate differences in treatment means.

RESULTS

Result of Survey

The responses for the questionnaire administered revealed that there are different types of fish species in the lake. Of note are the Tilapia family among which are *Tilapia zillii, Tilapia guineensis, Oreochromis niloticus*. Slight variations were also revealed in processing methods (data not shown). The different stages of processing by these processors (cropped unwashed fish) washed fish (washed, scalded, eviscerated and washed again); Salted fish; Salted fish in the smoking kiln; Salted fish in storage baskets) was noted for this study and this informed the stages at which samples were taken.

Physical changes of fish muscle observed along the processing line

In the first 30 minutes of smoking in stage IV, the temperature rose from 28.9^{o}C to 75^{o}C, the fish muscles were cooked and had a slightly brownish appearance. Further smoking intensified the brownish appearance of the smoked fish.

The mean physiochemical composition of water samples revealed that mean pH of the samples collected at the source was 7.1$\pm$ 1.0, while the ones collected at the middle of the lake and the edge were 7.4$\pm$1.7 and 7.5$\pm$ 0.8 respectively. The mean temperature recorded at the three sampling points was 29^{O}C. Total Dissolved Solids recorded at the middle of the reservoir (162ppm) was the lowest while 170ppm recorded at the edge was the highest. The dissolved oxygen of 4.8mg/l obtained at the edge was the highest, compared with the source (4.2mg/l) and mid-(4.0mg/l) (Table 1).

A significantly higher count ($P<0.05$) of (12.00$\pm$0.00CFU/g) was recorded in total aerobic plate count stage II (where fish was washed with water from the lake, scalded, eviscerated and washed again) when compared to unwashed fish muscle in stage 1 with 7.42$\pm$0.02CFU/g. The reverse was the case with the enterobacteriacea count from stage 1 to II.

Generally, sprinkling of fish with salt and heat from the smoking process significantly ($P<0.05$) decreased the microbial counts. However, a significant increase in counts was observed during storage) (Table 2). Furthermore a decrease in percentage moisture was observed along the processing line, while an increase was observed during storage. Percentage crude protein, fat, and ash increased along the processing line (Table 3).

In this study, only zinc, manganese and iron were significantly affected by heat along the processing line (Table 4).

DISCUSSION

It was observed that there was an increase in the moisture content of fish from stage I to II during the processing. This is as a result of scalding and washing. The percentage moisture decreased from 81.81 ± 0.03 at stage 1 to 26.41 ± 0.54 at stage 4 (the inception of the smoking process). This is due to the application of heat. The initial heating process which was very high (75^0C) led to the quick evaporation of moisture from the fish. The heating process resulted in a gradual reduction in moisture contents of the fish.

Smoking decreases the water activity in fish tissue as earlier reported [4, 5]. The percentage crude fat (lipids) increased on smoking. Similarly, Akande *et al*. [6] observed this when the lipid contents of mackerel increased on drying and smoking as a result of dehydration.

The crude protein in the mean proximate composition formed the largest quantity of the dry matter in all the fish samples. This is in line with the report of Daramola *et al*.[5] and Pannevis in smoked dried fish of different species [7] .

The result of the proximate composition showed an increase in the moisture content of the fish during storage. Hence, it may be deduced that the fish samples used in this study absorbed moisture during storage. This implies microbial multiplication which was encouraged by higher moisture contents. A report of an increase in moisture content with storage time had earlier been made [5].

The lower value obtained for crude fat in stages I-III when compared to IV-VII may be due to the oxidation of polysaturated fatty acids contained in fish tissue such as aldehydes, ketone and free fatty acid. However, Ebor *et al*.[8] noted that the increase in value obtained during smoking could be attributed to liquidification of the fat in the fish sample due to heat . An increase in the value of ash from stage IV-VII compared to that of stage I-III could be as a result of thermal effect on the elements contained in the fish sample.

The result of the nutritional composition shows that the higher moisture content, the lower the value of other nutritional components while the lower the moisture content,

the higher the values of other nutritional components. Earlier reports by Eyabi and Ningo & Daramola *et al.*[9] are in agreement with findings in this study [5].

Water activity determines the storage life of fish [5]. A gradual increase in moisture content was observed when the fish samples were stored for 3 days [stage 7, 8, 9]. This result corroborated the assertion of Daramola *et al.*[5] that there is an increase in the moisture content of the fish on storage . Hence, it can be deduced that tilapia fish absorb moisture during storage thereby predisposing it to early spoilage.

A significant increase was observed in the total aerobic plate count in stage II. This may be attributed to the higher microbial load of the water body and handling with contaminated hands. At this stage, the fish was scalded, eviscerated and washed with the water from the lake. A near similar report was made by Emikpe *et al*. [10] and Wogu & Maduakor who reported that all fish samples in their study were heavily contaminated with high indices greater than $13.07 \log_{10} cfu/g/cm^2$ [11]. Significantly higher microbial load was obtained in captured tilapia ($\log_{10}$ cfu/cm^2 13.20, $t = 3.369$; $p < 0.001$) than captured catfish. The decrease which was observed in the *Enterobacteriacea* counts was significant ($p<0.05$) while that of *Salmonella* counts showed no significant difference. This may be due to lower loads of enteric bacteria in the Eleyele Lake water at the time of study. The survivability of microbes in aquatic environment is determined by the conditions prevailing in the aquatic environment.

The reduction in the total aerobic plate count, *E. coli* count and Salmonella count from stage III-IV might be as a result of the increasing temperature from 28.9^0C to 75^0C. This could be attributed to thermal effects as indicated by Chang and Fang[12]. Such similar reduction in microbial count was observed when Heinitz and Johnson worked on the incidence of *Salmonella* species in smoked fish and shellfish [3].

The increase in values during storage may however be as a result of the rapid rate of multiplication of the microbes when exposed to the environment [13]. The usual practice of the reduction in the heat after one hour of smoking (between stages 5 and 6) led to a significant increase in the total aerobic plate count. A high total aerobic plate count implies that the fish product is of poor quality and predisposes consumers to health risks due to bacteria. This increase in total aerobic plate count may be as a result of a decrease in the temperature of samples which might encourage the multiplication of the microorganisms, Vishwanath *et al.*[14] noted this when he worked on the microbiological quality of smoked mud eel fish.

Application of salt at stage III retards the actions of these microbes temporarily when compared to stage II [14]. Salting is one of the oldest preservation methods to increase the shelf life of fish. [15]. Salt enhances flavour; and increases the perception of fullness, thickness and sweetness.

The levels of these heavy metals were not significantly different ($p>0.05$) before and after heat treatment for lead, cadmium, chromium, nickel, copper and magnesium. Low values were obtained for lead, cadmium, chromium, nickel, copper and

magnesium and no significant thermal effects were observed in these heavy metals and trace elements. FAO/APHCA [16] also noted that these are trace elements needed in micro quantities and thus accumulate in tissues in varying amounts. However, a significant reduction ($p<0.05$) was recorded for iron, zinc and manganese after smoking (stage 7). This could be attributed to the fact that heat has a significant effect on heavy metals by converting them into other compounds. A similar report of decrease in heavy metal was made by Ebor *et al*. [8]. . These heavy metals are elements needed in macro quantities in fish. Smoking reduced all elements except chromium which increased from 0.01±0.01ppm to 0.06± 0.01ppm. Generally, the elements occurred at low levels within international limits, thereby making this fish product safe for consumption [16].

CONCLUSION AND RECOMMENDATIONS

It has been noted that fish muscle tissue, which is widely consumed, may harbor pathogens because water for fish culture can harbor high microbial load including human pathogenic bacteria. Generally, the processing stages showed some variations in the microbial loads, nutritional contents and heavy metals or trace elements significantly ($p<0.05$) while the physicochemical changes in the water samples from source, middle and edge of the Eleyele river were not significantly different ($p<0.05$) except for total hardness and dissolved oxygen. The water from the lake which was used to clean the fish before smoking increased the total aerobic microbial load but decreased the enterobacteriacea counts significantly ($p<0.05$). Even though thermal effect on the smoked fish reduced the microbial count significantly initially, the usual reduction of the heat along the processing line gave way to a rapid multiplication of the microbes. Furthermore, the increase in the microbial load during the storage period of the smoked fish and increase in the chromium contents of fish after smoking which may result in toxicity if consumed should be of concern to public health. Improved hygiene in processing and lower storage temperature is, therefore, recommended.

Table 1: Physicochemical composition of water from different points along the lake

Parameters	Mid	Edge	Source
Ph	7.4±1.7[a]	7.5±1.3[a]	7.1±1.0[a]
Temperature (^{0}C)	29.0+ 0.30[a]	29.0+ 0.20[a]	29.0+ 0.30[a]
Total Dissolved Solids (ppm)	162.0+14.50[a]	170.0 + 21.91[a]	169.0 + 27.10[a]
Total hardness (gpg)	136.8+14.80[b]	102.6+15.62[a]	153.0 + 20.11[b]
Dissolved Oxygen (mg/l)	4.0+ 1.0[a]	4.8+ 0.4[b]	4.2+ 1.6[a]
Free chlorine	0.06+0.00[a]	0.05+ 0.01[a]	0.06+ 0.00[a]

Means in the same column followed by different superscript letters are significantly different ($p<0.05$).

Table 2: The mean value of microbial load present in the fish samples Log_{10} CFU/g

Samples	Total aerobic plate count	Enterobacteriacea count	Salmonella count
1	7.42±0.02[a]	9.45±2.55[a]	6.67±0.64[a]
2	12.00±0.00[b]	5.82±0.34[b]	6.54±0.20[a]
3	9.17±3.22[c]	5.13±0.28[b]	5.95±0.49[a]
4	8.44±2.56[a]	0.95±0.00[c]	0.95±0.00[b]
5	0.95±0.00[d]	3.28±2.33[d]	3.94±2.99[c]
6	9.56±2.44[c]	0.95±0.00[c]	0.95±0.00[b]
7	7.56±0.10[a]	2.98±2.03[d]	9.06±2.95[d]
8	12.0±0.00[b]	0.95±0.00[c]	9.16±2.84[d]
9	12.51±2.49[b]	2.49±013[d]	6.99±1.00[a]

Means in the same row followed by different superscript letters are significantly different ($p<0.05$).

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Table 3: The mean values of the % Proximate analysis of the fish samples

Sampled Stages	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fibre(%)	Ash(%)
I	81.81±0.03	13.63±0.06	0.91±0.04	0.05±0.01	7.08±0.40
II	83.41±0.03	14.17±0.06	0.90±0.02	0.05±0.01	6.71±0.04
III	82.19±0.06	13.63±0.06	0.7±0.03	0.07±0.05	6.51±0.08
IV	26.41±0.05	35.92±0.30	3.12±0.01	0.16±0.01	12.02±0.04
V	17.00±0.04	37.80±0.08	3.19±0.02	0.2±0.01	12.32±0.03
VI	14.65±0.47	36.33±0.21	3.16±0.08	0.14±0.03	12.21±0.18
VII	12.71±0.48	25.05±0.08	3.00±0.06	0.16±0.01	12.17±0.03
VIII	20.37±4.09	41.10±2.15	3.28±0.10	0.15±0.02	12.82±0.62
IX	21.92±5.97	44.69±315	3.45±0.23	0.2±0.09	16.03±1.74

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Table 4: Mean values of heavy metals and trace elements present in the fish samples

Heavy metal	Fish muscle samples before smoking	Fish muscle samples after smoking
Lead	0.08±0.01[a]	0.05±0.02[a]
Cadmium	0.05±0.00[a]	0.01±0.01[a]
Chromium	0.01±0.01[a]	*0.06±0.01[a]
Nickel	0.17±0.02[a]	0.10±0.01[a]
Copper	0.80±0.10[a]	0.07±0.01[a]
Zinc	55.55±0.15[a]	37.35±0.15[b]
Manganese	17.55±0.25[a]	11.4±0.10[b]
Iron	39.35±0.15[a]	28.4±0.10[b]
Magnesium	0.40±0.03[a]	0.27±0.02[a]

Means in the same column not followed by the same superscript letters are significantly different ($p<0.05$).

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