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EFFECTS OF TWO BIO-PRESERVATIVES ON KEEPING QUALITY OF AFRICAN MUD CATFISH, CLARIAS GARIEPINUS

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Abstract: **The study evaluated the effects of two commonly used household bio-preservatives, Sodium chloride and Ascorbic acid on the proximate and microbial compositions of smoked African mud catfish, Clariasgariepinus during a six weeks' storage period. Fresh fish samples weighing 450-500g each obtained from the fish farm of the Department of Animal and Environmental Biology, Adekunle Ajasin University, Akungba-Akoko, Nigeria were degutted and cleaned with water. A group of fresh fish sample was soaked in a mixture of 25% NaCl and 1% ascorbic acid for 1hour and 30min and then prepared for smoking while another untreated batch served as the control. The samples were smoked dried in the smoking kiln at 80^{o}C for 12hrs, cooled and packed in open and air tight containers in the laboratory for six weeks. The samples were then analysed for pH, proximate and microbial compositions at zero, two, four and six weeks respectively following standard procedures. There were significant differences ($p \leq 0.05$) between the treated and untreated groups. Pre-treatment of fish samples with a mixture of 25% NaCl and 1% ascorbic acid prior to smoking enhanced the keeping quality and stability of fish during storage.**

Keywords: **Bio-preservative, Ascorbic acid, Sodium chloride, Proximate, Microbial, Clariasgariepinus**

I. INTRODUCTION

Fish are important food resources worldwide due to their nutritional quality (FAO 2020). In developing countries, especially Nigeria, fish is the major source of animal protein, where it accounts for 75% of the daily animal protein, referred to as rich and the poor food as an important companion (Willet et al. 2019). As soon as fishes die, they become subject to post mortem changes and damage by microorganisms and insects that quicken the rate of fish spoilage, these together with inadequate preservation, processing and handling leads to the loss of over 40% of the total fish catch in Nigeria (Daramolaet al., 2007; Ime-Ibang and Fakunle, 2008; Akinwumi, 2011). Spoilage affects the odour, flavour, texture, colour and chemical composition of fish (Agbabiakaet al. 2012) and these in turn affect the nutritional quality, consumer acceptability and commercial value of fish (Daramolaet al. 2007). Fish processing helps to extend the storage life of fish and to give the product a form which is attractive to consumers (Tawari and Abowei, 2011). However, traditionally processed fish are still subject to many forms of loss or spoilage such as microbial spoilage, insect infestation and fragmentation (Abolagba and Nuntah 2011).
Preservation techniques are designed to inhibit the activity of spoilage bacteria and metabolic changes in order to prolong seafood quality (inhibiting spoilage and enzymes) as well as ensuring inhibition or inactivation of pathogens (Dehghaniet al. 2018). Some of the preservation techniques are affected through the control of temperature (by chilling or freezing),

reduction of water activity (drying, salting and smoking) and use of preservatives. Drying, salting and smoking are the most common preservative methods used in Nigeria. These processes ensure year round availability of fish and the distribution of wholesome fish products to all parts of the country. Drying dehydrates the fish and inhibits enzymatic action but during storage, nutritional quality may deteriorate as a result of lipid oxidation and microbial growth (Kumolu-Johnson and Ndimele 2011). The preservation method and mode of storage of preserved fish determines the rate and degree of fish spoilage or infestation. Therefore, storage methods that prevent lipid oxidation and microbial growth will inevitably increase shelf life of preserved fish.

The quality of smoked product is dependent on several factors including the quality of the fish at the time of smoking procedure employed (Da silva 2002). Consumers are discovering the good taste of smoked seafood, including smoked catfish. Smoke alone is insufficient for preserving food unless combined with another preservation method. Akinwumi (2014) observed that smoking demonstrated a better efficient method of fish processing in terms of the retention of protein value and reduction in the moisture content.

Salt and Ascorbic acid have been employed in the preservation of food commodities for ages. Common salt (NaCl) hinders the activity of bacteria, enzymes and chemicals in fish (Abolagba and Nuntah, 2011). Water is necessary for the microbial and enzymatic reaction involved in spoilage. Salt and Ascorbic acid preserve food by reducing the water in them.

It is well known that both enzymatic and microbial activities are generally influenced by temperature. Most bacteria are unable to grow at temperature below 10 degree Celsius and even psychotropic organisms grow very slowly, and sometimes with extended lag phases, when temperatures approach 0 degree Celsius (0°C). The shelf life of fish product, therefore, is markedly extended when products are stored at low temperature (FAO 2004).

Antimicrobial preservatives either present naturally or formed during processing or legally, intentionally added as ingredients, are capable of killing microorganism or controlling their growth in fish. Some antimicrobial preservatives used to prolong the shelf life of fishes are; acetic acid, polyphosphate, sodium chloride, and propionic preservatives.

Fish with high fat content is most susceptible to rapid deterioration and hence losses are encountered. Various preservation techniques have been utilized to improve the microbial safety and extend the shelf life of fish in general, including freezing, chemical preservation, salting and smoking (Nickelsonet al. 2001).

This study evaluated the effect of Salt and Ascorbic acid pretreatment on the proximate and microbial composition of dried C. gariepinus. According to Daramolaet al. 2007, the most important environmental factors governing the shelf life of fish are ambient temperature and humidity. Therefore, this study also assessed the shelf life of dried C. gariepinusstored differently at room temperature of (26-30^{o}C) for six weeks.

II. MATERIALS AND METHODS

Samples of catfish Clariasgariepinus were obtained from the fish farm of the Department of Animal and Environmental Biology and were taken to the departmental laboratory. The weights of the fish were measured using standard methods. The fresh fish were killed, degutted and washed. The sacrificed catfish sample were folded and randomly chosen and divided into two groups of 5 each per treatment, i.e treatment 1 (control: untreated samples) and 2 (treated samples). Fresh fish sample were kept in ice flakes to prevent deterioration and the second group was soaked in 25% NaCl and 1% ascorbic acid for 30 min and then prepared for smoking.

The treated fishes were laid on the different racks of a local-made metal oven. Two pots filled with burning charcoal and emitting constant heat were placed on the last rack of the metal oven. At intervals the fishes were turned to avoid burns and ensure uniform drying. The door of the oven was properly closed to retain heat and prevent contamination from dust and flies. The drying process took about 12 hours.

The dried fishes were allowed to cooled stored separately in open and airtight containers at room temperature in the laboratory. Five (5) fishes (untreated control) from each group were kept in different airtight plastic containers (closed containers), while another five (5) each (treated samples) were kept in plastic container with the open end covered with mushling clothe to prevent them from insects and other pests from set in. They were stored for the next six (6) weeks. The samples were then analysed in the laboratory for the proximate and microbial analysis for the period of zero, two, four and six weeks respectively.

Proximate Analysis

The dried fish stored in airtight containers were analyzed to determine the crude protein, crude fibre, fat, moisture and ash content. Proximate analysis was based on standard methods as described by AOAC (2005). Crude protein was determined using the Kjeldahl techniques. Ash content was determined by incinerating 1g of sample at 600^{o}c overnight.

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Microbial Analysis

Smoked samples were analyzed for the presence of pathogens after0, 2, 4, and 6 weeks of storage. 1g representative sample was obtained from the fish and was added to 9ml peptone solution then homogenized.1ml from the samples homogenate was transferred to 9ml of diluents to the required end point to generate lesser decimal dilution of the homogenate for counting and culturing techniques. Serial decimal dilutions of the 10^{-1} homogenate were done using peptone saline solution of the dilution to 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}.

1ml of the dilution 10^{-6} was plated in triplicate in different 20ml of molten clear agar at 37◦C for bacterial on nutrient agar.25◦c for fungi on potato dextrose agar. The plate was incubated appropriately for total mesophile aerobic quality (cfu/g).

Total plate count, coliforms, E. coli, Stapylococcus, Salmonella, yeast and mold counts were determined using a colony counter.

Statistical Analysis

Analysis of variance (ANOVA) was performed to the determine differences in physico-chemical properties among raw and treated catfish samples. The procedure General Linear Model (GLM) was used in data analysis. Tukey's studentized range test was performed for post-hoc multiple comparison.

III. RESULTS AND DISCUSSION

The mean values of the proximate composition of smoked catfish sample soaked in the solution (with 25% Nacl and 1% ascorbic acid) for 30 minutes are shown in Table 1. The data show that there were significant differences at ($p \leq 0.05$) between the treated and untreated groups. Water activity and pH are among the most critical factors affecting microbial growth and spoilage of foods. In this study the pH value of smoked control sample was 7.97%±0.01 and the pH of the treated samples was 7.47%±0.01. The fat content of smoked catfish varied from 16.47%±0.03. Fat content of the sample soaked in 25% sodium chloride and 1% ascorbic acid increased significantly to 17.86%±0.02 due to loss of moisture.

The protein content of the control sample ranged from 53.09%±0.02 while the protein content of the treated sample was 56.69%±0.01 showing that there were significant differences between them. Moisture content of the control sample was 14.53 0.03 while for the treated sample was 10.52%±0.02. This decrease was due to loss of water during smoking and due to the storage period. The ash content of the control sample was 9.39%±0.03 and that of the treated sample was 8.50%±0.02. For fibre the mean value of the control sample was 1.83%±0.03 while that of the treated was 1.38%±0.06 showing reduction in the fibre content. Mean value of carbohydrate content was 3.67%±0.01 while for the treated sample was 4.16%±0.01 showing an increase in the carbohydrate content.

Table 1: Proximate composition of smoked Clariasgariepinus treated with a mixture of 25% NaCl and 1% ascorbic acid at 30minutes post-treatment. Proximate parameters

*Mean with different superscript are significantly different at $p \leq 0.05$.

Treatment	Moisture Mean±S.E	Protein Mean±E	Ash Mean±S.E	Fat Mean±S.E	Fibre Mean±S.E	Carbohydrate Mean±S.E	pH Mean±S.E
Untreated smoked (treatment 1B)	14.53 ± 0.03^{h}	53.09 ± 0.07^{a}	9.39 ± 0.03^{g}	16.47 ± 0.07^{b}	1.83 ± 0.03^{g}	3.67 ± 0.01^{ab}	7.97 ± 0.01^{i}
Treated with 25% Nacl and 1% ascorbic acid for 30minutes.	10.52 ± 0.02^{c}	56.69 ± 0.01^{c}	8.50 ± 0.02^{c}	17.86 ± 0.02^{f}	1.38 ± 0.06^{c}	4.16 ± 0.01^{d}	7.47 ± 0.01^{h}
Anova (p=0.05)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
,SIG	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

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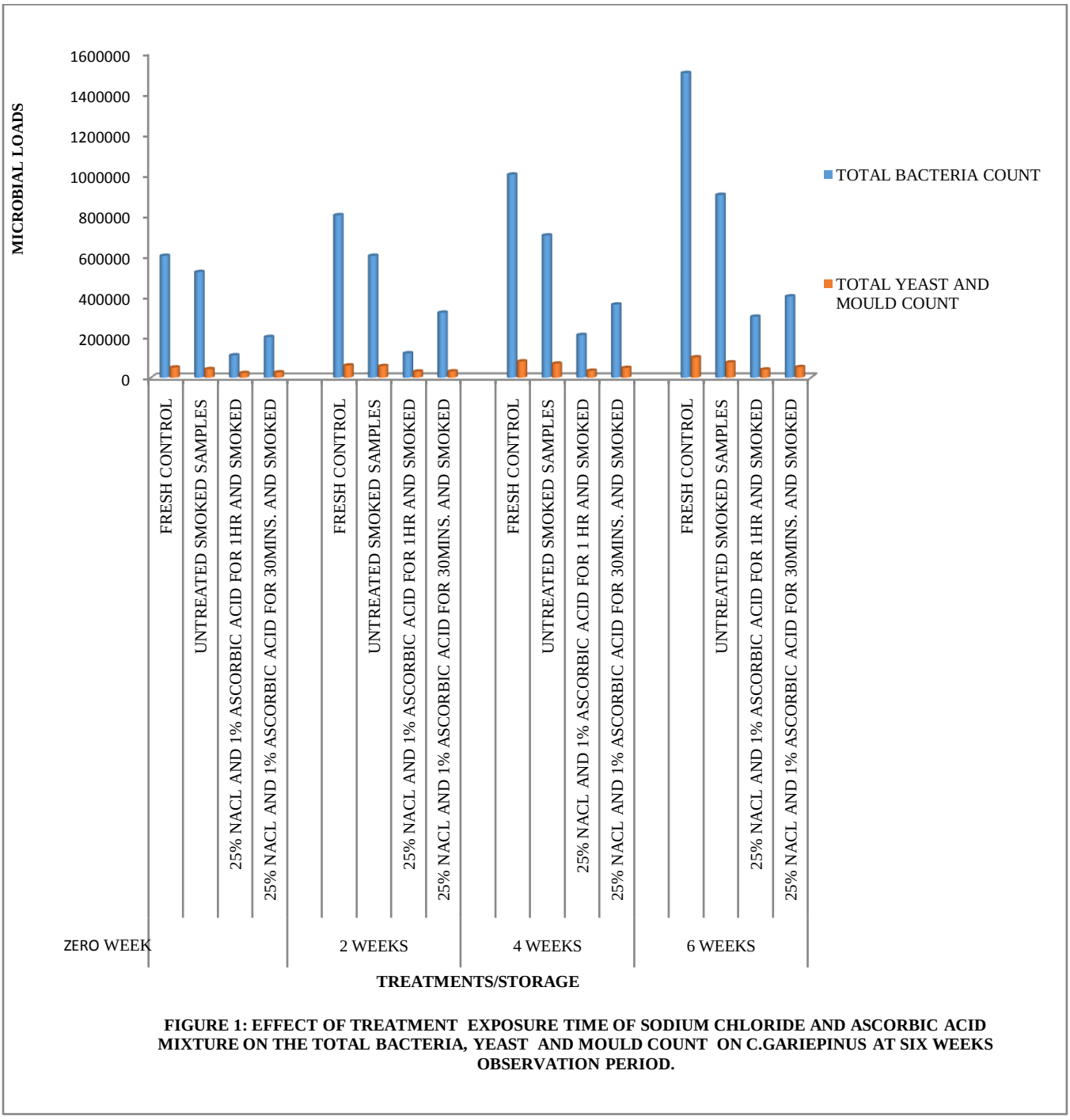

FIGURE 1: EFFECT OF TREATMENT EXPOSURE TIME OF SODIUM CHLORIDE AND ASCORBIC ACID MIXTURE ON THE TOTAL BACTERIA, YEAST AND MOULD COUNT ON C.GARIEPINUS AT SIX WEEKS OBSERVATION PERIOD.

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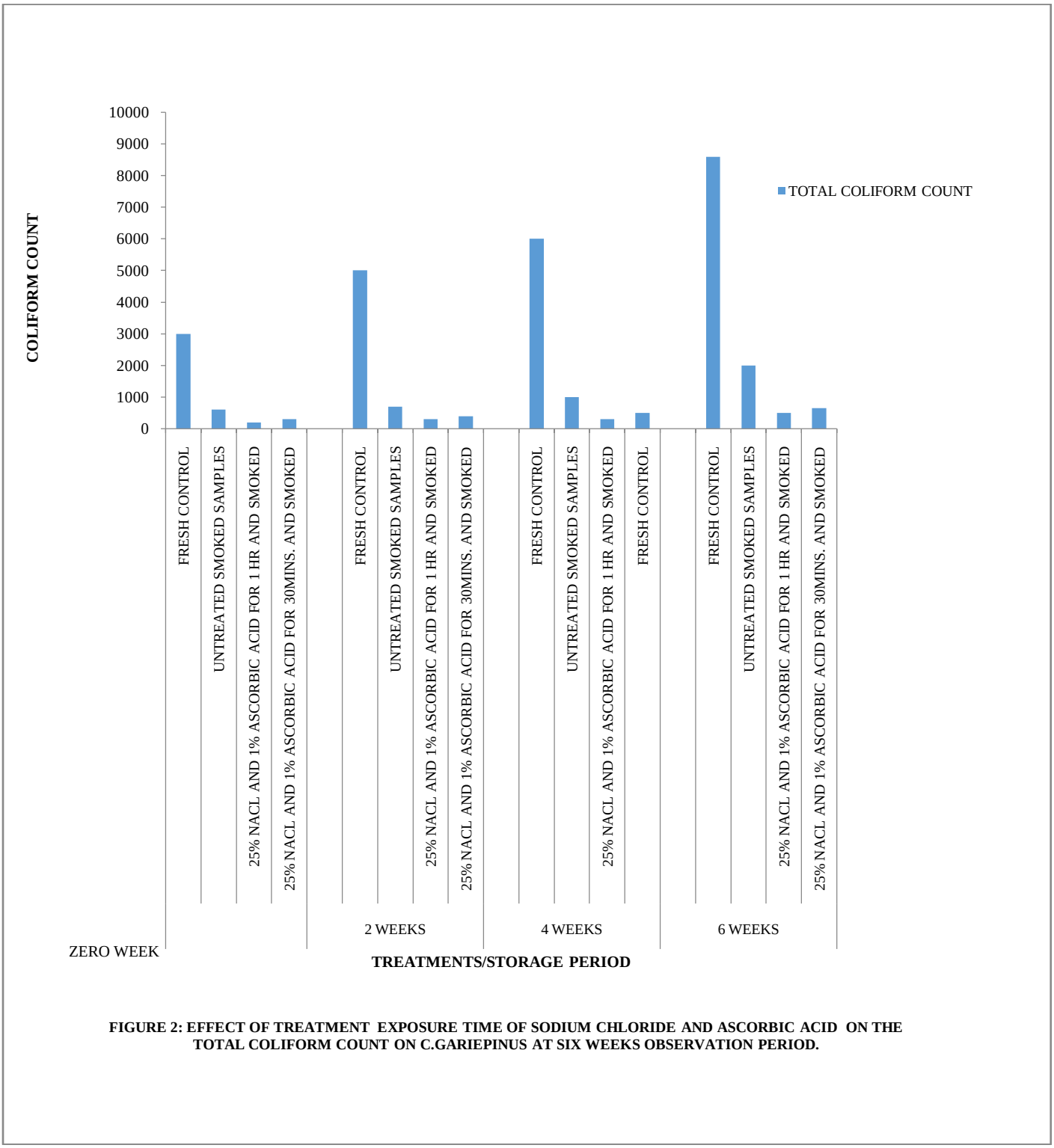

FIGURE 2: EFFECT OF TREATMENT EXPOSURE TIME OF SODIUM CHLORIDE AND ASCORBIC ACID ON THE TOTAL COLIFORM COUNT ON C.GARIEPINUS AT SIX WEEKS OBSERVATION PERIOD.

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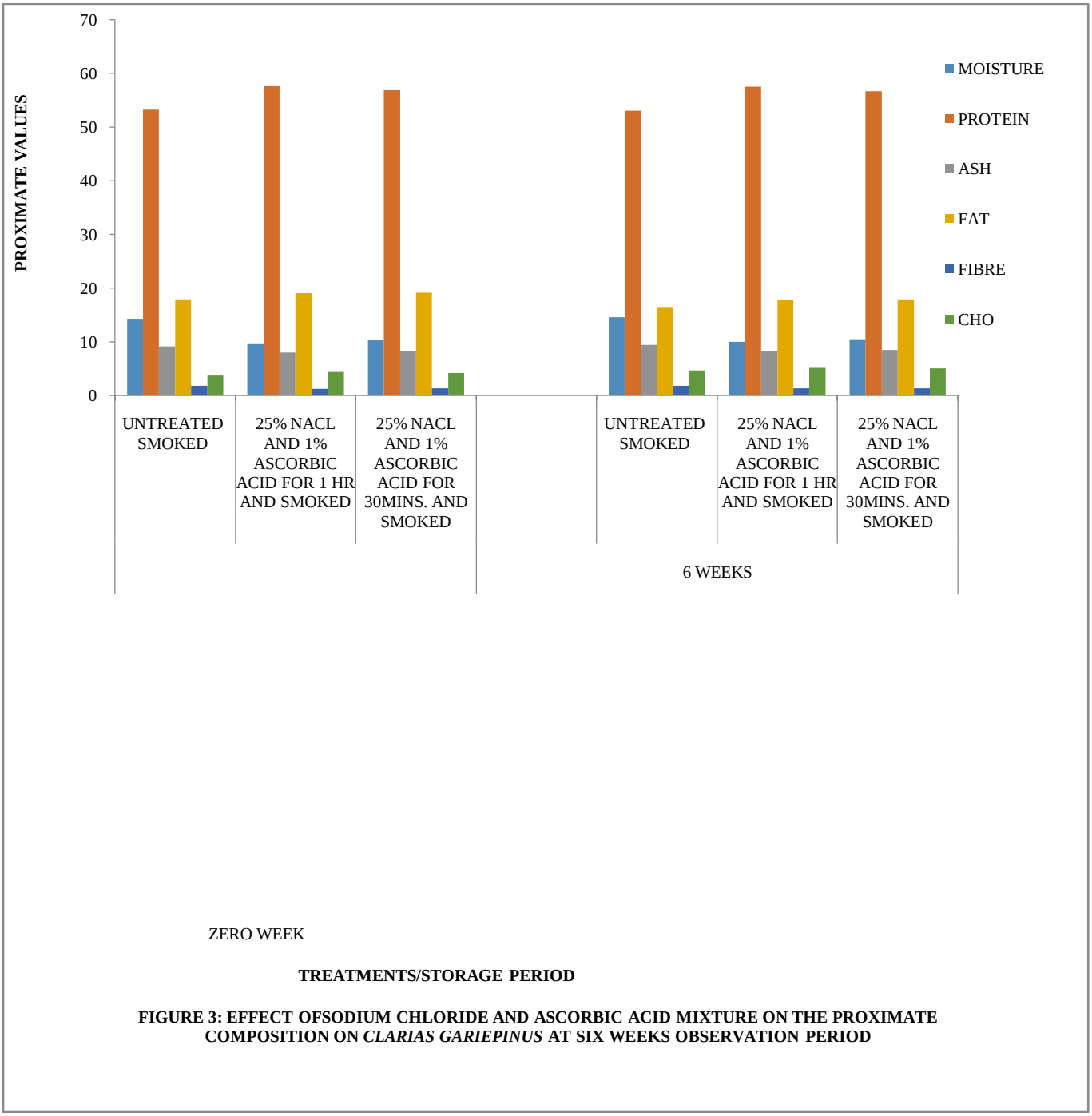

FIGURE 3: EFFECT OFSODIUM CHLORIDE AND ASCORBIC ACID MIXTURE ON THE PROXIMATE COMPOSITION ON *CLARIAS GARIEPINUS* AT SIX WEEKS OBSERVATION PERIOD

One of the primitive or oldest means of preservation of food product like fish is through smoking process. In this study, smoking reduced the moisture content of the fish sample. Moisture content in terms of water activity influences the microbial growth and hence, affects the stability of food during storage.

The proximate values for the untreated and treated samples were presented in figure 3. There were increase in the protein,

fat and carbohydrate values respectively in the treated samples when compared with the untreated one where decrease in values were recorded. There was significant increase in the moisture, ash, fibre and pH values of the untreated samples as opposed the treated samples where decrease in values were obtained. The moisture content observed from the untreated dictated the tune of microbial loads. There were statistically significant differences ($p<0.05$) among the fish samples alongside week 1-6 of storage time. This was possible because the fish were stored in cool dry places that encouraged increase in microbial growth and the non-re-drying of the fish for the period of storages as well encouraged the increase encountered in moisture content alongside storage time of the samples. The geographical increase in moisture of the fish samples with storage period observed was in agreement with the earlier reports by Ikutegbe and Sikoki (2014). There was increase in crude protein recorded for the treated fish samples with decrease in values recorded for the untreated samples. However, fish of lower protein values in the untreated samples could be due to microbial activities in the fish samples, thus the less value than the treated fish samples.

In the untreated sample which served as the control, increase in microbial load was observed in the total bacterial count, ($5.71\log_{10}$CFU/gto5.951 $\log_{10}$CFU/g), coliform ($2.77\log_{10}$CFU/gto$3.30\log_{10}$CFU/g),yeast and mould count ($4.62\log_{10}$CFU/gto$4.87\log_{10}$CFU/g) from zero week to six(6^{th}) week against the treated sample where significant reduction was noticed, the same observation was submitted by Salaudeenet al. (2010) who reported a significant differences at ($p\leq0.05$) between treated and untreated groups. The growth of microorganisms affects the proximate composition of fish which are pathogenic in nature. The mean value of the proximate composition of the smoked catfish samples treatment with 25% NaCl and 15 ascorbic acid compared with untreated smoked fish samples in table 1 shows that there are significant differences at ($p\leq0.05$) between treated and untreated groups.

Da silva (2002) earlier had reported that low water activity led to absence of mould, low microbial population and better shelf-stability where smoked fish were treated with 25% NaCl and 1% ascorbic,3% sodium lactate and other preservatives. The same observation was seen in the treated sample as the mean of the moisture content of the treated sample ranged from 10.52± 0.02 showing a reasonable reduction when compared to the mean of the moisture content of untreated sample (14.53±0.03) and this suggest the reason for reduction in the growth of microorganism (TPC, Coliform count and fungi) as shown in table 1 in the treated sample.

Physico-chemical analysis as shown in table 1, the pH ranged from 9.97±0.01 to 7.47±0.01 showing a decrease in the pH when treated with 25% NaCl and 1% ascorbic acid. The effect of this preservatives can't be overemphasized, as the reduction in pH of a medium limit the range of microorganism that thrive in such condition, hence microbes are pH specific and a drastic reduction in growth of microorganisms or microbial load is noticed in acidic pH. Thus pH is an important factor that affects microbial growth and spoilage of food and this may further help to explain the noticeable differences in the effects of antimicrobial and antioxidant (25% NaCl and 1% ascorbic acid) treatments on microbial population (Salaudeenet al. 2010).

Salaudeenet al. (2010) submitted that the understanding of microbial and physico-chemical attributes of the African catfish products may help to establish a generic Hazard Analysis and Critical Control Points (HACCP) plan to be used for species of catfish by evaluating the presence or absence of target food-borne pathogens, microbial population loads and physiological attributes of the smoked catfish.

Furthermore, Akinwumi and Adegbehingbe (2015) have revealed that the combination of smoking and treatments with antimicrobial agents and antioxidants such as 25%NaCl and 1% ascorbic acid retards microbial spoilage.

Several risk factors have been associated with microbial contamination of fish either during smoking process or cross contamination during storage. Serious concerns related to consumption of raw fish have been noticed or registered because of the presence of biological hazards (bacteria, virus, parasites and chemical biotoxins). Though hazard related to contamination could be controlled by hygienic practices and application of good manufacturing practice (Huss et al. 2000). Also Salihu-Lasisiet al. (2014) recommended that processed fish should be exposed to a drying temperature that will removed moisture content for the growth of microorganism.

IV. CONCLUSION

This study showed the effectiveness of preserving the nutritional attributes of fish by treating fish product/sample during storage. Specifically, the treatment of fish with 25% NaCl and 1% ascorbic acid could enhance preservation and stability of fish qualities during storage over a short period of time. Reduction in microbial load in the fresh fish was achieved during the smoking process and the reduction in moisture content of the experimental sample gave the smoked sample shelf stability.

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