Comparative Study on Graded Brine Solution and Spice Juice (*Allium sativium)* on the Nutritive Value and Shelf Life of *Clarias gariepinus*

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Abstract
A comparative study on graded brine solution and spice juice(Allium sativium) on the nutritive value and shelf life of Clarias gariepinus was undertaken. The experiment was conducted in a completely randomized design using 228 adult fish of C. gariepinus weighing 1000g each, which were distributed 15 fishes in each of the experimental units. The fish were distributed randomly to different treatment as assigned and all were replicated thrice. Treatment I and treatment 5 served as the control. Treatment 1 was 100% brine solution with 0% spice juice, Treatment 2 was 75% brine solution with 25% spice juice, Treatment 3 was 50% brine solution and 50% spice juice, Treatment 4 was 25% brine solution was with 75% spice juice and Treatment 5 was 0% brine solution with 100% spice juice. Samples were subjected to organo-leptic test, proximate composition analysis and microbiological analysis. There were significant differences (p<0.05) for taste in all the treatments except treatment 4 and 5. Appearance exhibit a significant difference (p<0.05) for all the treatments at day 21. Treatment 4 had the least moisture content at day 28. There were no significant differences (p>0.05) in ash content and fiber content. Treatment 4 had the best bacterial load at day 1 and also at the end of the storage period at day 28. Treatment 4 gave a better result in bacteria load and fungi growth, as well as the proximate composition analysis. It is recommended therefore that fish should be immersed in a mixture of 25% brine solution and 75% spice juice (Allium sativium) before smoking.

Keywords*: proximate composition, microbial load, spoilage, smoking, harvest.*

Introduction

Fish is an important source of protein to millions of people around the world. It is one of the cheapest sources of animal protein and other essential nutrients required in human diet (Sadiku and Olademeji, 1991). In many Asian Countries, over 50% of the animal protein intake comes from fish while in Africa the proportion is 17.5% (Williams *et al.*, 1998). Fish farming plays a very important role in achieving household and national food security in Nigeria, as well as poverty eradication (Salau *et al*., 2014). It is therefore sad to note that this important product had been greatly affected by post-harvest losses (Opara and Al-Jufaili, 2006), hence the need for an effective preservation and processing methods which is affordable, easily sourced and effective. Fish quality and quantity after harvest is heavily affected by deterioration and spoilage due to inadequate preservation. At present, there are numerous problems confronting fisheries and some of these have to do with the keeping quality of the produce (Okoro *et al.,* 2010).

Brine is a solution of salt (usually sodium chloride) in water; brine is used to preserve vegetables, fruits etc. Brine helps slow down and or prevents microbial spoilage and insect infestation. Spices on the other hand, are edible plant substances that possess anti oxidative, antiseptic and bacteriostatic properties (Onyeagbe, *et al.,* 2004). They are added to food to delay the onset of deterioration, such as rancidity, they also function as seasonings to the food as well as impact flavor to meals(Onyeagbe, *et al.,* 2004). Garlic (*Allium sativum*) is one of the frequently used natural ingredients to enhance flavour in food. It can function as an antibacterial, antifungal and has an anti-oxidative property. It also has a beneficial effect on the cardiovascular and immune system of man (Sallam, *et al.,* 2004).

This work was necessitated by the fact that preservation has remained a problem in harvest, to both capture and captive fisheries alike. The use of synthetic compound such as butylated, hydroxoyl toluene (BHT) commonly used as anti-oxidant in the food industry is not readily available to the local food and fish processors. Apart from its unavailability, Butylated hydroxyl toluene (BHT) and Butylated Hydroxyanosole (BHA) have been prohibited in many countries of the world because of their undesirable effect on the enzyme of human liver and lungs who consume food preserved with them (Sallam, *et al.,* 2004), this has paved way for the use of natural anti-oxidant such as spices which garlic is one of them. Utilization of locally available methods in fish preservation will go a long way in reducing wastage in the form of spoilage and take care of post-harvest losses as a result of bad processing and preservation. This will indirectly lead to increase in output, save cost and eventually lead to increase in revenue. This study therefore gives information on the nutritive value and shelf life of *Clarias gariepinus* when preserved with graded brine solution and spice juice.

Materials and Methods

The experiment was carried out in Michael Okpara University of Agriculture, Umudike (MOUAU),which lies between the latitude 5^0 26´N and longitude 7^0 33´E and a minimum temperature of 22^0C and altitude which falls within the range of (122m) 40ft above sea level. The fish for the experiment, African catfish (*C. gariepinus*) was purchased from the fish farm in the Department of Fisheries and Aquatic Resources Management (MOUAU), Umudike, Abia state.

The experiment was conducted in a completely randomized design using 228 adult fish of *C.gariepinus* weighing 1000g each. They were distributed 15 fish in each of the experimental units which were 15 in number. The fish were distributed randomly to different treatment as assigned and all were replicated thrice. Treatment I and treatment 5 served as the control. Treatment 1 was 100% brine solution with 0% spice juice, Treatment 2 was 75% brine solution with 25% spice juice, Treatment 3 was 50% brine solution and 50% spice juice, Treatment 4 was 25% brine solution and 75% spice juice and Treatment 5 was 0% brine solution with 100% spice juice. The fish were stunned using salt, decapitated and washed in clean water.

Fresh garlic (*Allium sativium*) was purchased from the local market in Umuahia, Abia State. The coats were removed, washed and crushed finely with a kitchen blender. The required quantity of 20g of garlic paste to 30cl of water was used (Kiin-kabari *et al*., 2011). It was mixed and filtered thoroughly. The filtrate was used as spice juice. Brine solution was prepared by dissolving 350g of salt (Sodium chloride) in one litre of water (Dobson, 1993). The different percentages of brine and spice juices required per treatment were calculated out and used to treat the fish.

The decapitated fish were then put in different bowls containing the various treatments and allowed for three hours in other for the fish to fully absorb the treatments. The already prepared fish was smoked with tropical hardwood using a smoking kiln.The smoked samples were cooled, packaged in a well labeled plastic buckets and stored at room temperature for the experimental period.

Samples were subjected to organo-leptic test, proximate composition analysis and microbiological analysis. Three fish were randomly selected for initial proximate composition analysis and microbiological test. These were repeated after smoking then weekly for the twenty eight storage days. Proximate composition analysis of the smoked fish was carried out according to the Association of Official Analytical Chemist (AOAC, 1990). The total coliform counts were determined according to the methods of fawole and Oso (1995). Evaluation of product quality was carried out by two trained panelist groups according to the method of poste *et al.* (1991). The qualities tested were; taste, texture and appearance.
The data collected were subjected to Analysis of Variance (ANOVA) and differences amongst means were separated using Duncan's Multiple Range Test.

Results and Discussion
The result of the organoleptic test analysis for taste is shown in table 1. There were significant differences ($p<0.05$) for taste in all the treatments except treatment 4 and 5. There were no significant different($p>0.05$) in day 14 between treatments 1 to 3. There were no significant differences ($p>0.05$) between treatment 4 and 5 throughout the days. In table 2, Appearance exhibit a significant difference ($p<0.05$) for all the treatments at day 21 with treatment 3 having the best appearance at day 7 and 14, while treatment 1 had the best appearance at day 21 and 28. Table 3 show organoleptic analysis for texture. There were no significant difference ($p>0.05$) between treatments 2 to 5 at day 7 and 14.

The result of the proximate composition analysis of the different treatments is shown in table 4. Treatment 4 had the least moisture content at day 28. There were no significant difference ($p>0.05$) in ash content. There were no significant difference ($p>0.05$) in fiber content, There were also no significant difference ($p>0.05$) in crude protein content except in treatment 5.
Table 5 shows the bacteria growth on nutrient agar amongst the treatments. There were no significant difference ($p>0.05$) in all the treatments except in day 7. There were significant differences ($p<0.05$) in treatments 2 and 3 in day 7 which were also significant different ($p<0.05$) from the rest of the treatments.

In table 6, there exists a significant different ($p<0.05$) between the initial and the storage days for all the treatments except in day 28. There were no significant difference ($p>0.05$) between day 1 and day 7 and also between day 14 and day 21. Treatment 4 had the best bacterial load at day 1 and also at the end of the storage period at day 28.

Table 7 shows the fungal growth on Potato Dextrose Agar (PDA) amongst the treatments. There were no significant differences ($p>0.05$) in all the treatments. However, treatments 3 and 4 had the least fungal growth all through.

Table 8, shows a significant difference ($p<0.05$) between the initial and the storage days for all the treatments up to day 21 except treatments 1 and 3. There were no significant difference ($p>0.05$) between the initial and day 28 for treatments 1 and 3. Treatments 2, 4 and 5 had the least fungi growth at the end of the storage period which also were not significantly different ($p>0.05$) from the preceding storage periods.

Figure 1 shows the number of occurrence and the various species of bacteria present. *Escherichia. Coli* and *Klebsiella* spp where absent after the application of the treatment. Figure 2 is the percentage occurrence of fungi and the various species present. *Fusarium* spp was absent after treatment while *Aspergillus* spp increases in percentage during storage.

Table 1.Organoleptic evaluation for taste

Days	Treatment				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Day 7	7.31±1.49[a]	5.10±2.64[ab]	4.80±2.30[b]	2.60±2.60[c]	2.70±0.95[c]
Day 14	5.00±1.76[a]	4.00±1.29[a]	4.20±1.40[a]	2.70±1.42[b]	2.20±1.14[b]
Day 21	5.00±0.67[a]	4.70±0.67[ab]	3.10±0.88[c]	3.20±1.48[b]	3.30±1.64[b]
Day 28	4.90±1.66[a]	4.60±2.17[ab]	3.20±0.91[b]	2.10±1.20[c]	2.30±1.89[c]

Values are means of treatment ± standard error of mean
Value with the same superscript within the same row not significantly different ($p > 0.05$)

Table 2. Organoleptic evaluation for appearance

Days	Treatment				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Day 7	4.90±2.81[a]	3.60±1.90[ab]	2.80±2.10[b]	2.00±1.41[b]	2.30±2.25[b]
Day 14	2.40±1.35[ab]	2.90±1.45[a]	3.50±127[a]	2.40±1.35[ab]	1.60±0.70[b]
Day 21	2.20±1.03[d]	2.60±0.97[cd]	3.10±0.57[c]	5.00±0.67[b]	6.90±1.10[a]
Day 28	2.70±1.16[b]	3.10±1.37[b]	3.00±1.41[b]	5.10±1.45[a]	6.20±1.93[a]

Values are means of treatment ± standard error of mean
Value with the same superscript within the same row not significantly different ($p > 0.05$)

Table 3.Organoleptic evaluation for texture

Days	Treatment				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Day 7	5.90±2.96[a]	3.90±2.69[b]	3.70±1.57[b]	2.10±1.20[b]	2.90±1.45[b]
Day 14	3.10±1.29[a]	3.30±1.16[a]	3.10±1.60[a]	2.70±1.94[a]	2.10±0.88[a]
Day 21	3.10±0.88[d]	2.20±1.30[d]	3.30±1.42[c]	5.10±0.74[b]	6.20±0.79[a]
Day 28	2.00±1.33[c]	2.10±0.88[c]	2.50±1.43[bc]	3.50±1.72[b]	5.70±1.42[a]

Values are means of treatment
Value with the same superscript within the same row are not significantly different ($p<0.05$)

Table 4: Proximate composition analysis of the treatments across the week

Treatments	Initial	Day1	Day 14	Day 28
Moisture Content				
Treatment 1	79.55±0.78^{a}	13.35±4.03^{b}	13.79±0.13^{b}	14.35±5.52^{b}
Treatment 2	79.55±0.78^{a}	14.28±2.72^{b}	12.52±2.32^{b}	18.98±1.03^{b}
Treatment 3	79.55±0.78^{a}	15.84±2.94^{b}	12.52±9.59^{b}	15.10±6.51^{b}
Treatment 4	79.55±0.78^{a}	14.40±5.26^{bc}	19.60±0.20^{b}	10.40±0.35^{c}
Treatment 5	79.55±0.78^{a}	13.28±0.40^{b}	12.20±2.40^{b}	10.98±3.78^{b}
Ash Content				
Treatment 1	12.10±0.14^{a}	5.05±3.89^{a}	9.30±6.22^{a}	11.10±0.71^{a}
Treatment 2	12.10±0.14^{a}	0.00±0.00^{c}	5.95±1.48^{a}	10.30±0.42^{a}
Treatment 3	12.10±0.14^{a}	4.90±0.14^{a}	8.85±6.86^{a}	6.10±6.36^{a}
Treatment 4	12.10±0.14^{a}	6.65±1.91^{a}	7.00±3.11^{a}	8.25±6.01^{a}
Treatment 5	12.10±0.14^{a}	10.85±0.49^{a}	11.05±2.19^{a}	8.35±3.61^{a}
Crude Fiber				
Treatment 1	0.00±0.00^{a}	2.79±1.11^{a}	2.00±0.00^{a}	2.50±0.71^{a}
Treatment 2	0.00±0.00^{a}	2.00±0.00^{a}	1.20±3.11^{a}	1.08±0.71^{a}
Treatment 3	0.00±0.00^{a}	5.22±0.30^{a}	2.56±1.20^{a}	3.32±0.45^{a}
Treatment 4	0.00±0.00^{a}	1.90±0.12^{a}	2.40±0.57^{a}	2.00±0.00^{a}
Treatment 5	0.00±0.00^{a}	0.53±0.75^{a}	0.60±1.13^{a}	1.00±0.00^{a}

Table 4: Cont.

Treatments	Initial	Day1	Day 14	Day 28
Ether Extract				
Treatment 1	10.15±0.28^{a}	19.24±5.47^{b}	12.63±3.25^{ab}	10.93±0.99^{c}
Treatment 2	10.15±0.28^{a}	19.24±5.47^{a}	10.12±7.23^{b}	8.30±0.41^{b}
Treatment 3	10.15±0.28^{a}	7.27±7.45^{a}	8.17±8.77^{a}	8.15±5.30^{a}
Treatment 4	10.15±0.28^{a}	9.40±9.01^{a}	9.24±1.65^{a}	9.60±0.71^{a}
Treatment 5	10.15±0.28^{a}	19.24±5.47^{a}	10.52±0.73	10.60±0.71^{b}
Crude Protein				
Treatment 1	66.88±0.03^{a}	64.30±1.95^{a}	64.83±0.37^{a}	64.83±0.37^{a}
Treatment 2	66.88±0.03^{a}	64.30±1.95^{a}	62.69±3.73^{a}	61.78±2.45^{a}
Treatment 3	66.88±0.03^{a}	69.32±32.88^{a}	63.01±1.41^{a}	64.32±2.47^{a}
Treatment 4	66.88±0.03^{a}	63.27±1.78^{a}	63.90±2.67^{a}	63.45±1.53^{a}
Treatment 5	66.88±0.03^{a}	60.78±1.48^{b}	61.69±1.18^{b}	62.31±1.11^{b}

Values are means of treatment
Value with the same superscript within the same row are not significantly different ($p<0.05$)

Table 5: Microbial load on Nutrient Agar (NA) amongst the treatments

Days	Treatment				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Day 1	$1.95x10^{4a}$	$1.75x10^{4a}$	$1.75x10^{4a}$	$1.75x10^{4a}$	$1.80x10^{4a}$
Day 7	$3.35x10^{4a}$	$2.60x10^{4b}$	$2.85x10^{4ab}$	$2.75x10^{4a}$	$2.75x10^{4a}$
Day 14	$5.20x10^{4a}$	$4.85x10^{4a}$	$4.85x10^{4a}$	$4.65x10^{4a}$	$5.35x10^{4a}$
Day 21	$6.65x10^{4a}$	$6.15x10^{4a}$	$5.90x10^{4a}$	$5.75x10^{4a}$	$6.60x10^{4a}$
Day 28	$9.5x10^{4a}$	$8.5x10^{4a}$	$9.60x10^{4a}$	$8.15x10^{4a}$	$8.75x10^{4a}$

Values are means of treatment
Value with the same superscript within the same row are not significantly different ($p<0.05$)

Table 6: Microbial load on Nutrient Agar (NA) across days

Treatments	Days					
	Initial	Day 1	Day 7	Day14	Day 21	Day 28
Treatment 1	$76.0x10^{3a}$	$19.5x10^{3c}$	$35.5x10^{3c}$	$52.0x10^{3b}$	$66.5x10^{3b}$	$95.0x10^{3a}$
Treatment 2	$76.0x10^{3a}$	$17.5x10^{3c}$	$26.0x10^{3c}$	$48.5x10^{3b}$	$61.5x10^{3b}$	$85.0x10^{3a}$
Treatment 3	$76.0x10^{3a}$	$17.5x10^{3c}$	$28.5x10^{3c}$	$48.5x10^{3b}$	$59.0x10^{3b}$	$96.0x10^{3a}$
Treatment 4	$76.0x10^{3a}$	$17.5x10^{3c}$	$27.5x10^{3c}$	$46.5x10^{3b}$	$57.5x10^{3b}$	$81.5x10^{3a}$
Treatment 5	$76.0x10^{3a}$	$18.0x10^{3c}$	$27.5x10^{3c}$	$53.5x10^{3b}$	$66.0x10^{3b}$	$87.5x10^{3a}$

Values are means of treatment
Value with the same superscript within the same row are not significantly different ($p<0.05$)

Table 7: Total Microbial Load on Potato Dextrose Agar (PDA) amongst the treatments

Days	Treatment				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Day 1	$1.0x10^{3a}$	$0.5x10^{3a}$	$1.0x10^{3a}$	$1.0x10^{3a}$	$0.5x10^{3a}$
Day 7	$1.5x10^{3a}$	$1.0x10^{3a}$	$1.5x10^{3a}$	$1.5x10^{3a}$	$1.0x10^{3a}$
Day 14	$1.5x10^{3a}$	$1.5x10^{3a}$	$1.5x10^{3a}$	$1.0x10^{3a}$	$1.5x10^{3a}$
Day 21	$2.0x10^{3a}$	$1.5x10^{3a}$	$2.0x10^{3a}$	$1.0x10^{3a}$	$1.0x10^{3a}$
Day 28	$2.0x10^{3a}$	$1.5x10^{3a}$	$2.5x10^{3a}$	$1.5x10^{3a}$	$1.5x10^{3a}$

Values are means of treatment
Value with the same superscript within the same row are not significantly different ($p<0.05$)

Table 8: Total Microbial Load on Potato Dextrose Agar (PDA) amongst days

Treatments	Days					
	Initial	Day 1	Day 7	Day 14	Day 21	Day 28
Treatment 1	3.5x10^{3a}	1.0x10^{3b}	1.5x10^{ba}	1.5x10^{3b}	2.0x10^{3a}	2.0x10^{3a}
Treatment 2	3.5x10^{3a}	0.5x10^{3b}	1.0x10^{3b}	1.5x10^{3b}	1.5x10^{3b}	1.5x10^{3b}
Treatment 3	3.5x10^{3a}	1.0x10^{3b}	1.5x10^{3b}	1.5x10^{3b}	2.0x10^{3a}	2.5x10^{3a}
Treatment 4	3.5x10 ^{3a}	1.0x10^{3b}	1.5x10^{3b}	1.0x10^{3b}	1.0x10^{3b}	1.5x10^{3b}
Treatment 5	3.5x10^{3a}	0.5x10^{3b}	1.0x10^{3b}	1.5x10^{3b}	1.0x10^{3b}	1.5x10^{3b}

Values are means of treatment
Value with the same superscript within the same row are not significantly different ($p<0.05$)

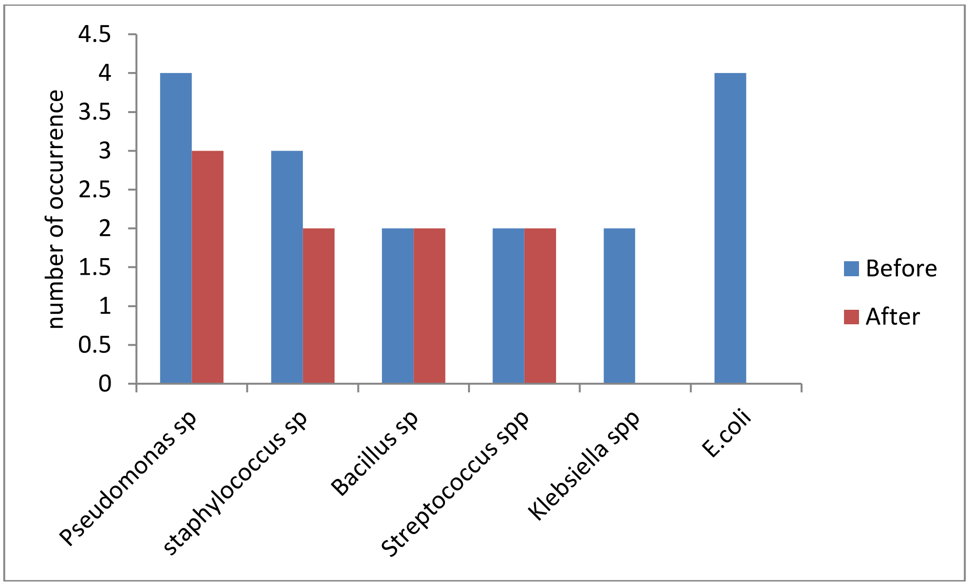

Fig 1: Bacteria occurrence before and after treatment

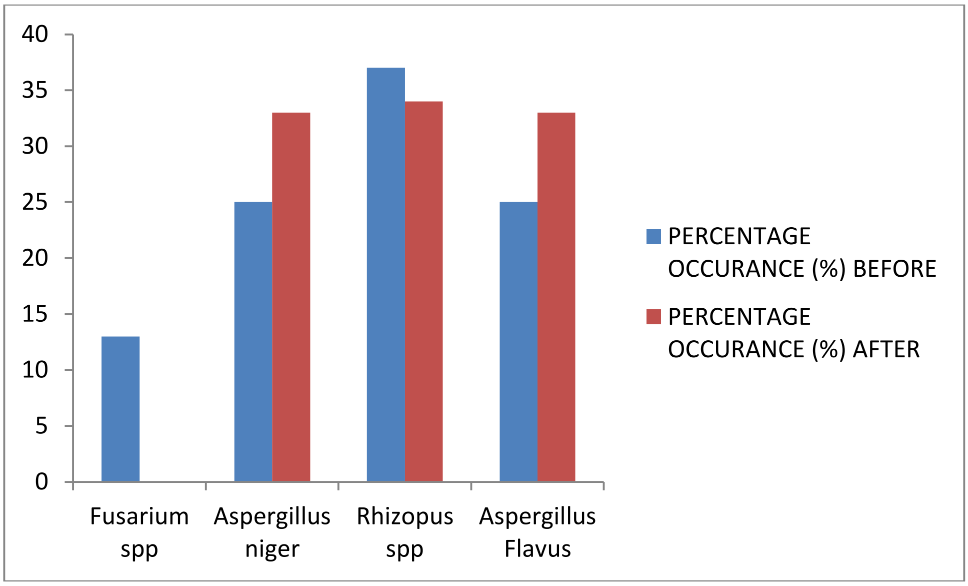

Fig 2: percentage fungal occurrence before and after treatment

Food preservatives and anti-oxidants are used to prolong the shelf-life of food by killing micro-organism or control their growth in food. They also preserve by preventing or retarding the oxidative deterioration of food (Kumola – Johnson and Ndimele, 2011).The results of this study showed that brine solution and spice juice significantly enhance the texture, taste and appearance of the fish. They also extend the shelf life of the fish. The drying effect during smoking together with the antioxidant and bacteriostatic effects of the smoking allow smoked products to have extended shelf-life (Eyo, 2001). It was observed that treatment with higher percentage of spice juice had more shelf life, better in appearance, texture, and taste then those with lower percentage of spice juice. Storage time had no effect on the organolleptic taste and general acceptance of fish treated with higher percentage of brine solution and spice juice. This might be due to the duration of the study (28 days), although, sallam *et al.* (2004), obtained a similar result in their study in which they examined the effect of galic in chicken sewage for 21 days.

The reduction in the moisture content could be attributed to exposure of the fish to heat. This is supported by Kumola – Johnson and Ndimele (2011), who reported a reduction in moisture content during hot smoking. The observed differences amongst the treatments in moisture content may also be due to the variation in the moisture absorbing properties of the various combinations of brine and spice juices used. This is in line with the observation of Fapohunda and Ogunkoya (2006).Also, there was a drop in the protein content of the fish in the course of the experiment. This reduction of protein content could be due to the action of enzymes and heat which could alter or denature the protein content of the fish, as well as the slight increase in fiber contents impacted by the treatments.

The result on microbiological analysis shows that all the treatments behaved alike in the preservation of the fish. There was a slight increase in day28 though the effect was not significant which shows that the products can be stored up to a month without a drastic effect in its quality. The quality and storage time can also be improved upon by the use of more desired storage materials and storage at reduced and or controlled temperature. This is in line with Kolodziejska *et al.* (2002) who reported a decrease in the shelf life of processed stored seafood and recommended that in other to maintain its quality at storage, storage temperature and packing methods are necessary factors to consider for longer storage time. There was also an increase in microbial load with storage time. This result is in agreement with the work of Bilgin *et al.* (2008) as well as Hood *et al.*(1983) who both reported that microbial load increases with duration of storage and temperature.

E. coli were detected in the fresh fish samples. It is found mostly found in fresh animal meat and fresh fish especially in polluted sources. This result showed that preservatives played an inhibitory effect on the smoked fish. According to Zaika (1988) inhibitors may reduce the levels of microbial growth in foods. The absence of *E. coli* and *Fusarium* spp. on the treated samples throughout the storage period could be due to the inhibitory effects of garlic.

Conclusion

This work has shown that the mixture of brine solution and spice juice (*Allium sativum*) had chemical preservative and anti, oxidative properties. It has also shown that garlic spice juice can enhance flavor in Food, has a high spectrum of actions which include antibacterial, anti-fungal and anti-oxidative function which can also benefits the cardiovascular and immune system of human beings. Brine on the other hand has been effective in preserving fish as it prolonged shelf-life and kills off micro-organisms. This study however, is of a great relevance because of its socio-economic importance to farmers and masses in general. Treatment 4 gave a better result in both bacteria load and fungi growth, as well as the proximate composition analysis. It is recommended therefore that fish should be immersed in a mixture of 25% brine solution and 75% spice juice (A*llium sativium)* before smoking.

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