Original Research Article

EFFECT OF SOME PRESERVATIVES ON THE MICROBIAL QUALITY OF COLD-SMOKED CATFISH AT REFRIGERATED TEMPERATURE.

ABSTRACT

Aim: This study investigated the preservative effect of potassium sorbate, ginger, garlic, and a blend of, ginger and garlic on the microbiological quality and shelf stability of cold smoked Catfish (*Clarius gariepinus*) at refrigerated storage.

Methodology: One hundred (100) of the freshly harvested catfish obtained from the MOUAU Fish Farm were processed and divided into five lots of 20 each. Four marinating baths containing 0.5% potassium sorbate, 3% ginger, 3% garlic, and a blend of 1.5% ginger and 1.5% garlic respectively were prepared. Each catfish lot except the fifth lot (which served as a control) was marinated for 2 h and cold smoked for 8 h. The samples were then stored for fourteen days at refrigeration temperature (4 ± 2 °C during which analyses were carried out. The Total viable count, Total fungal count, and bacterial counts (*Escherichia coli, Salmonella, Staphylococcus sp, and Listeria sp*) of the catfish samples before, during, and after storage were analyzed.

Result: The total viable count (TVC) was reduced from $8.7x10^5$ to $1.2x10^5$ CFU/g, 1.13 $x10^6$ to $2.4x10^5$ CFU/g, 1.09 x 10^6 to 2.00 $x10^5$ CFU/g, 9.60 $x10^5$ to 1.50 $x10^5$ CFU/g, and $1.19x10^6$ to $3.00x10^5$ CFU/g for 3% ginger, 3% garlic, 1.5% ginger +1.5% garlic, 0.5% potassium sorbate, and the control at refrigeration storage respectively. All the treatments were effective against *E. coli, Listeria monocytogen, and Salmonella count. Similarly, the* 3% of ginger treatment recorded counts of $1x10^5$ CFU/g for *Staphylococcus* counts, during refrigeration storage. Furthermore, the total fungal count decreased for all the treatments.

Conclusion: The study found that cold smoked catfish treated with 0.5% potassium sorbate, 3% ginger, 3% garlic and a blend of 1.5% ginger and 1.5% garlic can be stored for 14 days at refrigeration storage provided the storage condition is maintained.

Keywords: Microbial Quality, Refrigerated Condition, Ginger, Garlic, Potassium Sorbate, Bacterial Count.

1. INTRODUCTION

Fish has several benefits that make its desirability for food and business. It is a main source of animal protein, constitutes an important component of the diet for many people, and supplies the much-required nutrients for healthy living. Fish protein now takes precedence over other proteins of animal origin, and compares favorably with that of milk, egg, and meat in its amino acid composition [1]. Magawata and Shina [2] reported that “Fish is a highly nutritious food and it is particularly valued for its protein of high quality better than those of meat and eggs”. Fish contain very high-quality oil and fats and contains a good amount of thiamine, riboflavin, minerals, phosphatides sterols, enzymes, hormones, hydrocarbons, and pigments [3].

Fish is highly perishable. As important as it is to healthy living and for income generation, one of the greatest challenges facing fish consumption and business in Nigeria is poor or inadequate post-harvest processing and management. It is for this level of perishability that fish is processed into smoked or canned fish, and other fish-based products [5].

In Nigeria, one of the historical ways of preserving fish is smoking. Smoking is carried out in fishermen camps in chambers of traditional kilns made of clay, cement blocks, or drums, using salt as a main preservative. Technically, the moisture content of fish products is determined by the duration of smoking. The rate of microbial growth and invariably the shelf life or keeping qualities of the fish have been found to be determined by the moisture (or water activity) of a smoked fish [5]. Gram [6] observes that microbes e.g., *Listeria. monocytogenes* have been found in crabmeat samples and smoked fish samples. Da Silva [7] also states that these microbes are ubiquitous in nature and able to grow at low temperatures and in high salt concentrations of up to 10%. Hence, preservation by drying is affected by lowering the fish's water activity to a level at which micro-organisms can no longer grow [8]. In addition, the use of salt as a preservative is associated with certain health challenges, resulting in the need to utilize alternative and natural spices such as ginger, garlic, etc.

Unlike the traditional fish-smoking method, cold smoking is not common in Nigeria. In this method, the temperature of the smoke does not exceed 30°C [8]. According to Oyesele [9], cold-smoked samples had the highest moisture content in comparison with the moisture content of hot-smoked and oven-dried tilapia samples. This is because the cold-smoked fish is not fully dehydrated, hence they do not last long. Studies including [8] have compared cold-smoked and hot-smoked fish and observed that cold-smoked fish possess higher nutritive value than hot-smoked fish because the latter became cooked in the process.

There has been a growing need to find an alternative to salt as a preservative in the smoking of fish, as well as to improve the shelf life of cold-smoked fish. These preservatives (ginger and garlic) contain spectra of biologically active compounds that have medicinal effects [10,11]. Therefore, this study assesses the preservative effect of 3% ginger, 3% garlic, a blend of 1.5% ginger and 1.5% garlic, and

0.5% potassium sorbate on the microbiological and shelf stability of cold-smoked catfish at refrigerated temperature.

2 MATERIALS AND METHODS

2.1 Sample Collection

One hundred (100) samples of Catfish (*Clarias gariepinus*) with weights ranging between 300 to 400 g were used. The Catfish samples were purchased from the Michael Okpara University of Agriculture, Umudike's fish farm, while the ginger, garlic, and other ingredients were purchased from Ubani main market, Abia State. They were taken to the Laboratory prior to analyses for pre-smoking treatment and thereafter to the College smoking kiln for smoking.

2.2 Processing and Preparation Operation:

2.2.1 Preparation of ginger, garlic and potassium sorbate solution

Fresh garlic and ginger solution were prepared by the method described by [12]. Five grams (5g) of potassium sorbate powder was dissolved into 1000 ml of distilled water to obtain 0.5% potassium sorbate solution.

2.2.4 Fish Processing and smoking

One hundred (100) pieces of the Catfish were cut open through the head down to remove the guts and gills, washed with running tap water, and placed in five (5) lots of 20 pieces each. Following marinating standards (WHO), the ratio of fish to liquid was 1:1 weight per volume of fish samples. Each of the lots was treated with 3% ginger, 3% garlic, 1.5% ginger, and 1.5% garlic, and the 4th lot with 0.5% potassium sorbate, respectively, as brine for 2 h before they were conveyed for cold smoking at a temperature around 28°-32 ^{o} C. The lots of folded catfish were arranged on the smoking racks and subjected to cold smoking for 8 h. At intervals of 1 h, the smoking temperature was monitored using a thermometer.

2.2.5 Sample preparation for analysis

After smoking, the fish samples were cooled and separately stored in five labelled metal baskets each containing 20 pieces of the cold-smoked fish. The samples of cold-smoked *Clarias gariepinus*, were coded based on the different treatments they were subjected to. The cold-smoked fish samples were carried to the Department of Food Science and Technology, Michael Okpara University of *Agriculture*, Umudike laboratory for further microbial analyses. Storage of the fish samples was in refrigeration temperature and wrapped with foil for 15 days.

The preparation of the sample for microbial analysis was done as described by [13]. For the entire experiment 1ml of appropriate dilution was used for inoculation on Tryptone soy agar and the selective media for the isolation of *Escherichia coli, Staphylococcus spp, Listeria*, *Salmonella spp*, and fungi. This experiment was carried out on the fish samples in alternate days (0, 2, 4, 6, 8,10,12,14 days) making 8 testing days for microbial analysis.

2.3 Experimental/ Research Design

The experiment was modelled according to [14]. The study was laid out on a two-factor (A x B) factorial experimental setting, with factors A and B being the preservatives and the storage period, respectively. With exception of the microbiological studies, the factor levels were generally set at 5 x 3 for the preservatives and storage period, respectively. The microbiological study was expanded to a 5 x 8 factor level setting, to monitor the effectiveness of the preservatives for a longer period.

2.4 Microbial Analysis

The fish samples were subjected to microbial analysis to determine the microbial load of the samples. Selective media were used for the isolation of the specific pathogenic organisms which include; Sorbitol MacConkey agar (CM0813) for *Escherichia coli*, brilliance *Listeria* agar base (CM1080) for *Listeria* spp, brilliance *Salmonella* agar base (ISO16140 standard) for *Salmonella* spp, Dichloran Bose-Bengal Chloramphenicol (DRBC) Agar base (Oxoid), for yeast and mould, Baird Parker agar base (Oxoid) for *Staphylococcus* spp, peptone water (CM009) for the dilution and tryptone soy agar which was used for total bacteria count (TBC). These media were prepared according to the manufacturer's instructions. The microbial load for the cold smoked fish samples was recorded in CFU/g.

2.4.1 Enumeration of *Escherichia coli* /Coliforms

Sorbitol MacConkey agar (CM0813) was prepared according to manufactures instruction. Cefixime-Tellurite supplement (SR0172E Oxoid) was used for the isolation of *Escherichia coli*. Exactly 1ml of the aliquot of the samples made up in the peptone water (in triplicate per sample) was poured into the plate and about 15 – 20 ml of molten agar (45-50 ^{o}C) was then poured into the plate, mixed thoroughly and allowed to solidify, inverted and incubated at 37^{o}C for 24 h. Pink colonies were observed in the plate and was counted using colony county indicating the presence of *Escherichia coli* /Coliforms.

2.4.2 Enumeration of *Listeria monocytogenes*

Brilliance *Listeria* agar base (CM1080) was prepared according to manufacturer's instruction. One vial of Brilliance *Listeria* selective supplement (SR0227E) and Brilliance *Listeria* differential supplement (SR0228E) were added for the isolation of *Listeria spp*. Approximately 15 – 20 ml of molten agar was poured into the plate mixed thoroughly and allowed to solidify, 1ml of the aliquot was inoculated into the *Listeria* agar plate, spread with sterile glass rod spreader, inverted and incubated at 37 ^{o}C for 24 h. Green colonies were observed and counted with colony counter which indicated the presence of *Listeria* spp.

2.4.3 Enumeration of *Salmonella* spp

Brilliance *Salmonella* agar base (CM1092) was prepared according to the manufacturer's instruction. One vial of Brilliance *Salmonella* selective supplement (SR0194E) was added for the isolation of *Salmonella* spp. purple and blue colonies were counted and recorded with colony counter, indicating the presence of *Salmonella* spp (*Salmonella typhimurium* and *Klebsiella pneumonia*.

2.4.4 Enumeration of *Staphylococcus aureus*

Baird parker agar (with egg yolk Tellirite Emulsion, Oxoid), which was prepared according to the manufacturer's instruction. The plates were incubated at 37 ^{0} C for 24 h. Black colonies with halo around it was observed and counted using colony counter indicating the presence of *Staphylococcus* spp**.**

2.4.5 Enumeration of yeast and mould

Dichloran Bose-Bengan Chloramphenicol (DRBC) Oxoid was used for the isolation of yeast and mould. DRBC was prepared according to the manufacturer's instruction. The mixture was homogenized and 0.1

ml of the aliquot was inoculated on the DRBC plate and incubated for 25 ^{0}C for 5 days. The presence of yeast and mold was counted and recorded in CFU/g of the sample.

2.5 Statistical Analysis

Data obtained from bench analyses were recorded and subjected to statistical analysis of variance (ANOVA) using SPSS version 20.0 statistical package for windows (SPSS, Chicago IL). Differences were considered to be significant at ($p<0.05$), means were separated using Duncan's multiple range test.

3. RESULT AND DISCUSSION

The Catfish samples were subjected to analysis immediately after cold smoking on the day of production (i.e., day 0). The fish was stored in the refrigerator at (4 ± 1^{O}C**)** for fourteen (14) days. Samples were also taken for microbial analysis in alternate days of the storage periods (i.e., day 2, 4, 6, 8, 10, 12 and 14). The specific microorganisms that were evaluated in the cold smoked fish samples during the storage period exhibited their defined colony characteristics on the respective selective media; *Escheriachia coli* appeared as pink colonies, *Staphylococcus* sp had black colouration with shinny halo around it, *Listeria monocytogenes* as green colonies, *Salmonella* spp as purple and blue colonies while the yeast and mould as pink colonies.

3.1. Effect of Preservatives and Cold Smoking on Total Viable Count (CFU/g) for Catfish stored at Refrigeration Temperature.

It was observed that the initial total viable count of all the cold smoked fish before storage day 0 was low, with the lowest count obtained in the 3% ginger-treated sample (8.5×10^{5} CFU/g) and the highest number found in the untreated sample (1.19×10^{5} CFU/g) as shown in Table 1. This could be attributed to the treatment given to the fish samples prior to cold smoking which must have destroyed / inhibited some of the bacteria present in the fish. On storage day 2, there was a significant increase in the number of total viable counts in all the samples with 1.5% garlic+ 1.5% ginger recording the least increase (1.09×10^{6} – 1.52×10^{6} CFU/g) and the highest in the untreated sample (1.19×10^{6} – 2.22×10^{6} CFU/g). However, after day 2 of the observed increase, there was a constant and consistent decrease in the number of total viable counts across the samples as the duration of storage increased, although the number of counts

was higher in the untreated sample and thus, they are significantly different ($p<0.05$) from the treated samples. This observed decrease could be a result of the moisture in the fish having been converted to ice and at this temperature, only the psychrotrophic organisms survived and also due to the preservative effect of the preservatives. This is in agreement with Oyesele [9], who reported a decrease in the number of bacteria as the storage period increased in the cold-smoked Tilapia under cold storage conditions. This indicated that the microbial composition of the fish samples changed with the time of storage.

Table 1 Effect of Preservatives and Cold Smoking on Total Viable Count (CFU/g) of Catfish stored at Refrigeration (4 ± 1°C) temperatures.

Duration of storage (Days)	Preservatives					Duration of storage main effect
	Untreated	**3% Ginger**	**3% Garlic**	**1.5%Ginger + 1.5%Garlic**	**0.5%-K-Sorbate**	
0	$1.19x10^{6ijk}$	$8.7x10^{5n}$	$1.13x10^{6jkl}$	$1.09x10^{6klm}$	$9.6x10^{5pqr}$	**$9.5x10^{5d}$**
2	$2.22x10^{6a}$	$2.01x10^{6b}$	$1.81x10^{6bc}$	$1.52x10^{6def}$	$1.63x10^{6cd}$	**$1.84x10^{6a}$**
4	$1.82x10^{6bc}$	$1.54x10^{6de}$	$1.44x106^{defgh}$	$1.37x10^{6efghi}$	$1.25x10^{6hijk}$	**$1.48x10^{6b}$**
6	$1.47x10^{6defg}$	$9.4x10^{5lmn}$	$1.22x10^{6ijk}$	$1.25x10^{6hijk}$	$1.29x10^{6ghijk}$	**$1.23x10^{6c}$**
8	$1.31x10^{6fghij}$	$7.3x10^{5no}$	$1.11x10^{6jklm}$	$8.1x10^{5n}$	$9.1x10^{5mn}$	**$9.7x10^{5d}$**
10	$9.3x10^{5lmn}$	$4.4x10^{5pqrs}$	$5.5x10^{5op}$	$4.4x10^{5pqrs}$	$3.8x10^{5pqrst}$	**$5.5x10^{5e}$**
12	$5.4x10^{5opq}$	$2.0x10^{5tu}$	$4.7x10^{5pqr}$	$3.2x10^{5qrstu}$	$2.5x10^{5stu}$	**$3.5x10^{5f}$**
14	$3.0x10^{5rstu}$	$1.2x10^{5u}$	$2.4x10^{5stu}$	$2.0x10^{5tu}$	$1.5x10^{5u}$	**$2.0x10^{5g}$**
Preservative main effect	**$1.22x10^{6a}$**	**$8.7x105^{cd}$**	**$9.9x10^{5b}$**	**$8.7x10^{5c}$**	**$7.9x10^{5d}$**	

Values are means from replicated determinations: n= 16 for preservative main effect; n= 10 for duration of storage main effect; n= 2 for the interactions. Means bearing different superscripts in the duration of storage main effect column, preservative main effect row and the interaction cells are significantly ($p<0.05$) different.

Result obtained from the preservative main effect shows the effect of preservatives on total viable counts as it relates to the shelf stability of the samples. A significant difference ($p<0.05$) between the treated and untreated samples was observed. However, 0.5% potassium sorbate treated sample was most effective in reducing the total viable count (7.9 $x10^5$ CFU/g), followed by 3% ginger (8.6$x10^5$ CFU/g), 1.5% ginger and 1.5% garlic (8.7$x10^5$ CFU/g), and 3% garlic (9.9 $x10^5$ CFU/g) was the least among the treated samples. This result shows that the preservatives used were effective in reducing the number of total viable counts and slowing down the rate of deterioration of the fish sample hence extending the shelf stability of this product compared to the untreated sample. This study disagrees with the findings of Mosarrate [15], who reported an increase in TVC during refrigeration storage of salt +garlic treated smoked dried *Mastacembelus Pancalus* (biam).

The effect of storage on total viable count as it relates to shelf stability of the cold smoked samples shows a significant difference ($p<0.05$) in the storage days except for day 0 and day 8 storage days which have no significant difference between themselves but are significantly different ($p< 0.05$) among the other days. However, the highest count was on day 2 (1.84$x10^6$ CFU/g) before the moisture in the fish was converted to ice, while the lowest was recorded on day 14 (2.0$x10^5$ CFU/g). This shows that storage in refrigeration condition of spice-treated cold smoked catfish significantly decreased the total viable count probably because of the preservatives applied and hence extended the shelf life of the samples.

3.2 Effect of Preservatives and Cold Smoking on Total Fungal Count (TFC) count (CFU/g) of Catfish stored at Refrigeration (4 ± 1^{o}C) temperatures.

The values of the total fungal count (TFC) obtained from Table 2 clearly shows that there were significant variations ($p<0.05$) among the samples. However, all the treated samples were significantly lower than the untreated sample except on day 0. Three percent (3%) of ginger had the lowest number of counts from day 0 till the end of the storage period and the untreated sample was the highest from day 2 all through the storage period. This indicated that 3% ginger is highly effective against fungal in cold smoked fish. The effect of preservatives on the total fungal count from the preservative main effect shows that the sample treated with 3% ginger (1.4$x10^5$ CFU/g) was most effective in slowing down the total fungal count,

followed by 1.5% ginger + garlic ($1.7x10^5$ CFU/g), 0.5% potassium sorbate ($1.8x10^5$ CFU/g) then 3% garlic ($2.4x10^5$ CFU/g). Values obtained in this result indicated that these samples treated with preservatives are effective against fungal loads and are significantly different from the untreated samples ($3.4x10^5$ CFU/g). This result agrees with Idris *et al.* [19] who reported that different concentrations of ginger significantly reduced fungal load on smoked dried catfish and Negbenebor *et al.* [16] who used clove and ginger individually and in combination to reduce the fungal load of smoked fish.

The effect of storage on the Total fungal count from the duration of storage main effect shows a significant variation ($p<0.05$) in the storage days. The total fungal count at day 0 was high in all the samples but as the duration of storage increases the number of the total fungal counts was decreasing as well. However, at day 10 storage there was no detectable number of total fungal count in the treated sample ($<10^1$ CFU/g) till the end of the storage period. This could be attributed to the effect of the preservatives on the TFC, which means that storage at frozen temperature has significant effect on the total fungal count.

Table 2 Effect of Preservatives and Cold Smoking on Total Fungal Count TFC (CFU/g) of Catfish stored at Refrigeration (4 ± 1°C) temperatures.

Duration of storage (Days)	Preservatives					Duration of storage main effect
	Untreated	3% Ginger	3% Garlic	1.5%Ginger + 1.5%Garlic	0.5%-K-Sorbate	
0	$5.7x10^{5ab}$	$4.6x10^{5abc}$	$6.1x10^{5a}$	$5.8x10^{5ab}$	$5.7x10^{5ab}$	**$5.6x10^{5a}$**
2	$4.3x10^{5abcdef}$	$4.3x10^{abcd}$	$4.1x10^{5abcde}$	$2.6x10^{5cdefghi}$	$1.9x10^{5efghij}$	**$3.4x10^{5b}$**
4	$3.7x10^{5bcdefgh}$	$2.7x10^{5cdefghi}$	$2.8x10^{5cdefghi}$	$2.3x10^{5cdefghij}$	$1.6x10^{5ghij}$	**$2.6x10^{5bc}$**
6	$3.0x10^{5cdefghi}$	$<10^{1j}$	$1.4x10^{5hij}$	$1.7x10^{5fghij}$	$1.5x10^{5ghij}$	**$1.5x10^{5d}$**
8	$2.2x10^{5defghij}$	$<10^{1J}$	$1.1x10^{5abc}$	$1.3x10^{5ij}$	$1.5x10^{5bcdefg}$	**$2.4x10^{5cd}$**
10	$1.3x10^{5ij}$	$<10^{1j}$	$<10^{1j}$	$<10^{1j}$	$<10^{1j}$	**$2.5x10^{4e}$**
12	$<10^{1j}$	$<10^{1j}$	$<10^{1j}$	$<10^{1j}$	$<10^{1j}$	**$<10^{1e}$**
14	$<10^{1J}$	$<10^{1J}$	$<10^{1J}$	$<10^{1J}$	$<10^{1J}$	**$<10^{1e}$**

Preservative main effect	**$3.4x10^{5a}$**	**$1.4x10^{5c}$**	**$2.4x10^{5ab}$**	**$1.7x10^{5bc}$**	**$1.8x10^{5ab}$**

Values are means from replicated determinations: n= 16 for preservative main effect; n= 10 for duration of storage main effect; n= 2 for the interactions. Means bearing different superscripts in the duration of storage main effect column, preservative main effect row and the interaction cells are significantly ($p<0.05$) different.

That increase in storage days leads to a decrease in the total fungal count of treated cold smoked catfish. The significance of yeast and mould in the food according to Fraizer and Westoff [20] is that the metabolites of these fungi are mycotoxins and in recent times, it has raised a serious concern because of their carcinogenic properties. The syndrome resulting from the consumption of toxins in mold-contaminated food is known as mycotoxicosis.

3.3 Effect of Preservatives and Cold Smoking on *Escherichia coli* (CFU/g) of Catfish stored at Refrigeration (4 ± 1^{o}C) temperatures.

The values obtained from Table 3 shows that there was little or no trace of *Escherichia coli* on the fish samples at day 0 as the values were below 10^{1} CFU/g. On day 2, the number of *Escherichia coli* increased significantly ($p<0.05$) compared to the number at the beginning of the storage across the samples. Further significant decrease in the number of *E. coli* was observed as the storage duration increases. This also could be attributed to effect of preservatives on the *E. coli* present in the treated fish samples. However, throughout the storage period, the *E. coli* count was higher in the untreated sample than in the treated ones and are said to be significantly different ($p<0.05$) from each other. From the preservative main effect, the effect of preservatives on the *E. coli* in the spice treated cold smoked catfish shows significant difference ($p<0.05$) between the treated and the untreated sample from the Table 3. However, 3% ginger was more inhibitory to *E coli* ($8.0x10^{4}$ CFU/g) followed by 3% garlic ($1.3x10^{5}$ CFU/g) and 0.5% potassium sorbate ($1.3x10^{5}$ CFU/g), then 1.5% ginger + garlic ($1.5x10^{5}$ CFU/g) This result shows that the preservatives used are effective against *E. coli.* This work agrees with the report of Ezeama and Ozua [17] and Jeyakumar [21] who opined that ginger and garlic have known antimicrobial qualities in food products.

The effect of storage on the presence of *E. coli* from the duration of storage main effect shows a significant difference ($p< 0.05$) in the *E. coli* during the storage duration. The number of the count on days 0, 10, 12 and 14 ($<10^1$, 5.6×10^4, $<10^1$ and $<10^1$ CFU/g) respectively is significantly different from day 2 ($3.0x10^5$ CFU/g) and days 4, 6, and 8 ($2.3x10^5$, $1.9x10^5$, and $2.1x10^5$ CFU/g) respectively. This result reveals that storage duration significantly reduced the *E coli* across the samples and that the longer the storage duration, the lower the *E. coli count.* Therefore, refrigeration storage reduced the number of *E. coli* found in the fish samples significantly from day 6 thus extending the shelf stability of the spice-treated cold smoked catfish for at least 8 days and beyond the 14^{th} day the samples were stored. The presence of this pathogen in food / fish causes serious health challenges like severe diarrhea which can lead to renal damage and other serious complication including death Ezeama [13].

Table 3: Effect of Preservatives and Cold Smoking on *Escherichia coli* count (CFU/g) of Catfish stored at Refrigeration (4 ± 1^{o}C) temperatures.

Duration of storage (Days)	Preservatives					Duration of storage main effect
	Untreated	3% Ginger	3% Garlic	1.5%Ginger + 1.5%Garlic	0.5%-K-Sorbate	
0	$<10^{1g}$	$<10^{1g}$	$<10^{1g}$	$<10^{1g}$	$<10^{1g}$	**$<10^{1c}$**
2	$4.3x10^{5a}$	$2.7x10^{5bcdef}$	$3.1x10^{5abcd}$	$1.8x10^{5cdef}$	$3.3x10^{5abc}$	**$3.0x10^{5a}$**
4	$3.6x10^{5ab}$	$2.5x10^{5bcdef}$	$2.1x10^{5bcdef}$	$1.6x10^{5defg}$	$1.9x10^{5cdef}$	**$2.3x10^{5b}$**
6	$2.8x10^{5abcde}$	$1.3x10^{5efg}$	$2.2x10^{5bcdef}$	$1.4x10^{5efg}$	$1.8x10^{5cdef}$	**$1.9x10^{5b}$**
8	$2.6x10^{5bcdef}$	$<10^{1g}$	$1.9x10^{5cdef}$	$1.2x10^{5abcd}$	$1.2x10^{5abcd}$	**$2.1x10^{5b}$**
10	$1.6x10^{5defg}$	$<10^{1g}$	$1.2x10^{5fg}$	$<10^{1g}$	$<10^{1g}$	**$5.6x10^{4c}$**
12	$<10^{1g}$	$<10^{1g}$	$<10^{1g}$	$<10^{1g}$	$<10^{1g}$	**$<10^{1c}$**
14	$<10^{1g}$	$<10^{1g}$	$<10^{1g}$	$<10^{1g}$	$<10^{1g}$	**$<10^{1c}$**
Preservative main effect	**$1.9x10^{5a}$**	**$8.0x10^{4b}$**	**$1.3x10^{5b}$**	**$1.5x10^{5b}$**	**$1.3x10^{5b}$**	

Values are means from replicated determinations: n= 16 for preservative main effect; n= 10 for the duration of storage main effect; n= 2 for the interactions. Means bearing different superscripts in the duration of storage main effect in column, preservative main effect in row and the interaction cells are significantly ($p<0.05$) different

3.4. Effect of Preservatives and Cold Smoking on *Staphylococcus* count (CFU/g) of Catfish stored at Refrigeration (4 ± 1^{o}C) temperatures.

The result presented in Table 4 reveals that at day 0, 3% ginger recorded the lowest number of *Staphylococcus* count (< 10^1 CFU/g) and the highest number in 3% garlic ($4.4x10^5$ CFU/g) although there was a significant difference ($p< 0.05$) with the number of counts in all the samples. However, at day 2, 3% garlic dropped by one count ($4.3x10^5$ CFU/g) and was still higher than, in the other samples whereas 0.5% potassium sorbate recorded the lowest number of counts. As the duration of storage was increasing the number of counts of *Staphylococcus* sp decreased in all the samples. It was also noted that *Staphylococcus* count in the treated samples were much lower than in the untreated sample, particularly from day 6 storage days.

The effect of preservatives on the *Staph* count from preservatives main effect shows variation among the treatments with 3% ginger ($1.1x10^5$ CFU/g) and 0.5% potassium sorbate ($1.2x10^5$ CFU/g), not significantly different ($p<0.05$) from each other, 3% garlic ($1.7x10^5$ CFU/g) and 1.5% ginger + garlic ($1.6x10^5$ CFU/g) with same superscript not significantly different ($p>0.05$) between themselves but significantly different ($p< 0.05$) among other treatment. This shows that those preservatives used were effective against *Staphylococcus* but at varying levels.Therefore, the preservative-treated samples gave a better result and lower number of *Staphylococcus* than the untreated sample.

However, the effect of storage on the number of staph counts from storage main effect indicated a significant difference ($p<0.05$) in the storage duration. It was observed from the table below that at day 2 ($3.3x10^5$ CFU/g) the number of counts was higher compared to the number at the beginning of storage ($2.8x10^5$ CFU/g) but with increase in the duration of storage, there was a decrease in the number of *Staphylococcus.*

Table 4: Effect of Preservatives and Cold Smoking on *Staphylococcus* count (CFU/g) of Catfish stored at refrigeration (4 ± 1^{o}C) temperatures.

Duration of storage (Days)	Preservatives					Duration of storage main effect
	Untreated	**3% Ginger**	**3% Garlic**	**1.5%Ginger + 1.5%Garlic**	**0.5%-K-Sorbate**	
0	$3.5x10^{5bc}$	$<10^{1m}$	$4.4x10^{5a}$	$3.1x10^{5bcde}$	$2.8x10^{5cdef}$	**$2.8x10^{5b}$**
2	$2.6x10^{5defg}$	$3.3x10^{5bcd}$	$4.3x10^{5a}$	$3.8x10^{5ab}$	$2.4x10^{5efghij}$	**$3.3x10^{5a}$**
4	$2.4x10^{5efghi}$	$2.5x10^{5defgh}$	$1.8x10^{5ghijkl}$	$2.8x10^{5cdef}$	$1.8x10^{5ghijkl}$	**$2.3x10^{5c}$**
6	$2.1x10^{5fghijk}$	$1.7x10^{5hijkl}$	$1.6x10^{5ijkl}$	$2.1x10^{5\ fghijk}$	$1.5x10^{5jkl}$	**$1.8x10^{5d}$**
8	$2.1x10^{5fghijk}$	$1.4x10^{5kl}$	$1.3x10^{5kl}$	$1.2x10^{5\ l}$	$1.2x10^{5l}$	**$1.4x10^{5e}$**
10	$1.6x10^{5ijkl}$	$<10^{1m}$	$<10^{1m}$	$<10^{1m}$	$<10^{1m}$	**$3.2x10^{4f}$**
12	$1.2x10^{5l}$	$<10^{1m}$	$<10^{1m}$	$<10^{1m}$	$<10^{1m}$	**$2.3x10^{4f}$**
14	$<10^{1m}$	$<10^{1m}$	$<10^{1m}$	$<10^{1m}$	$<10^{1m}$	**$<10^{1f}$**
Preservative main effect	**$1.9x10^{5a}$**	**$1.1x10^{5c}$**	**$1.7x10^{5ab}$**	**$1.6x10^{5b}$**	**$1.2x10^{5c}$**	

Values are means from replicated determinations: n= 16 for preservative main effect; n= 10 for duration of storage main effect; n= 2 for the interactions. Means bearing different superscripts in the duration of storage main effect in column, preservative main effect in row and the interaction cells are significantly ($p<0.05$) different.

This again could be attributed to the effect of the preservatives on *Staphylococcus* sp and also the moisture having been converted to ice which would have aided the multiplication of *Staphylococcus* in the samples. These findings have agreed with the findings of Oyesele [9] who dictated bacteria counts at the beginning of storage experiment in cold smoked Tilapia at cold storage condition but subsequently, during the last week of storage found no trace of bacteria specie on the sample. *Staphylococcus aureus* is a bacterium that causes staphylococcal food poisoning, a form of food gastro enteritis through the production of enterotoxins. It is resistance to freezing and survives well in food stored at – 20^{0} C [22].

3.5 Effect of Preservatives and Cold Smoking on *Listeria* sp count (CFU/g) of Catfish stored at Refrigeration (4 ± 1°C) temperatures.

Result obtained in this study indicated that at day 0, *Listeria* sp was not found in all the samples. However, on day 2 they emerged. Their emergence at day 2 could mean that the phenolic compound in the wood smoke inhibited its growth but subsequently the spore of this organism germinated being a psychrotrophic organism. The untreated sample had the highest count of *Listeria* sp ($4.9x10^5$ CFU/g) and the lowest was found in 3% garlic ($<10^{-1}$ CFU/g). It was also observed that as the duration of storage was increasing, the number of *Listeria* sp count was decreasing in all the samples. This decrease could be attributed to the preservative effect of the preservatives on the fish against *Listeria* growth. The effect of preservatives on *Listeria* sp from the preservative main effect shows that 3% ginger ($1.5x10^5$ CFU/g) was most effective in inhibiting Listeria spp followed by 3% garlic and 1.5% ginger + garlic ($1.6x10^{5b}$, $1.6x10^{5b}$ CFU/g) respectively then 0.5 % potassium sorbate ($1.7x10^5$ CFU/g) though there was no significant difference (<0.05) among the treatments.

Table 5: Effect of Preservatives and Cold Smoking on *Listeria* sp count (CFU/g) of Catfish stored at Refrigeration (4 ± 1°C) temperatures.

Duration of storage (Days)	Preservatives					Duration of storage main effect
	Untreated	3% Ginger	3% Garlic	1.5%Ginger + 1.5%Garlic	0.5%-K-Sorbate	
0	$<10^{1i}$	$<10^{1i}$	$<10^{1i}$	$<10^{1i}$	$<10^{1i}$	**$<10^{1d}$**
2	$4.9x10^{5b}$	$3.9x10^{5bcd}$	$<10^{1i}$	$2.7x10^{5cdefg}$	$3.4x10^{5cde}$	**$3.0x10^{5a}$**
4	$4.1x10^{5bc}$	$2.9x10^{5cdefg}$	$3.4x10^{5cdef}$	$2.6x10^{5defgh}$	$3.9x10^{5bcd}$	**$3.4x10^{5a}$**
6	$3.2x10^{5cdef}$	$2.3x10^{5efgh}$	$2.6x10^{5defgh}$	$3.6x10^{5bcde}$	$3.1x10^{5cdefg}$	**$3.0x10^{5a}$**
8	$3.2x10^{5cdef}$	$1.7x10^{5gh}$	$1.9x10^{5fgh}$	$2.4x10^{5efgh}$	$2.3x10^{5efgh}$	**2.3x105b**
10	$2.4x10^{5efgh}$	$1.2x10^{5hi}$	$1.5x10^{5bcde}$	$1.7x10^{5gh}$	$1.2x10^{5hi}$	**$1.6x10^{5b}$**

12	$<10^{1l}$	$<10^{1l}$	$1.5x10^{5a}$	$<10^{1l}$	$<10^{1l}$	**$1.3x10^{5c}$**
14	$<10^{1l}$	$<10^{1l}$	$<10^{1l}$	$<10^{1l}$	$<10^{1l}$	**$<10^{1d}$**
Preservative main effect	**$2.2x10^{5a}$**	**$1.5x10^{5b}$**	**$1.6x10^{5b}$**	**$1.6x10^{5b}$**	**$1.7x10^{5b}$**	

Values are means from replicated determinations: n= 16 for preservative main effect; n= 10 for duration of storage main effect; n= 2 for the interactions. Means bearing different superscripts in the duration of storage main effect column, preservative main effect row and the interaction cells are significantly ($p<0.05$) different.

The untreated sample which had the highest number of counts of *Listeria* ($1.7x10^5$ CFU/g) is significantly different ($p<0.05$) from the treated sample. The result above suggested that the different preservatives used were effective in inhibiting the growth of *Listeria* sp in the treated cold smoked catfish under refrigeration storage conditions

The result obtained from the storage main effect shows a significant difference ($p<0.05$) in the storage days with storage day 0 ($<10^{-1}$) and day 14 ($<10^{-1}$) having no count followed by day 12 ($1.3x10^5$ CFU/g), day 10and 8 ($1.6x10^{5b}$, $2.3x10^5$ CFU/g) respectively then days 6,4,2 ($3.0x10^5$, $3.4x10^5$ and $3.0x10^5$ CFU/g) respectively. Although, during the storage period it was observed that *Listeria* sp emerged but the population was decreasing as the storage days increases. This could be attributed to the antimicrobial effect of the preservatives applied, on *Listeria*. Therefore, spice treatment on cold smoked catfish can inhibit/reduce the population of *Listeria* sp under cold storage conditions thus reducing the risk of pathogenic infection on cold smoked fish and prolonging the shelf life of the fish samples. According to Ezeama [13] and Lianou and Sofos [23], the prevalence of *Listeria monocytogenes a* pathogenic organism is of public health concern as its presence in food can cause an illness known as Listeriosis and the mortality rate is as high as 20 -30%.

3.6. Effect of Preservatives and Cold Smoking on *Salmonella* sp count (CFU/g) of Catfish stored at Refrigeration (4 ± 1^oC) temperature

From Table 6 below, it was observed that at day 0 before storage began, no amount of *Salmonella* was found in all the samples ($<10^1$ CFU/g) but as the storage commenced, on the day 2 the sample treated

with 3% ginger had ($5.3x10^5$ CFU/g) as the highest number of growth and the lowest number was found in the sample treated with 0.5% potassium sorbate ($2.8x10^5$ CFU/g). Moreover, as the storage days were increasing the population of *Salmonella* sp was also decreasing significantly across the samples till the last day of storage day 14. However, it was noted that the rate of decrease was much slower in the treated samples than in the untreated when they are compared. This means that the treated samples had better results than the untreated sample. The effect of preservatives on *Salmonella* sp from the preservatives' main effect statistically, shows no clear significant difference (< 0.05) with respect to the individual effect of these preservatives on *Salmonella* sp among the samples. Though, in the last four days of the storage, the samples treated with 3% ginger, 3% garlic, and 0.5% potassium sorbate had no trace of the organism whereas the untreated sample even till the last day of storage had some growth on them. The effect of storage on *Salmonella* sp shows a significant difference ($p<0.05$) in the storage days from the storage main effect. This indicated that storage has an effect on *Salmonella* though the organism may not be present on the samples immediately after cold smoking but during storage, it may appear but as the storage days increase under cold storage the number of this organism would be reduced significantly especially in the treated samples.

Incidences of *Salmonellosis* (a food poisoning syndrome), typhoid, and paratyphoid fever are indications of the consumption of foods contaminated with *Salmonella* [13, 20].

Table 6: Effect of Preservatives and Cold Smoking on *Salmonella* sp count (CFU/g) of Catfish stored at Refrigeration (4 ± 1°C) temperatures.

Duration of storage (Days)	Preservatives					Duration of storage main effect
	Untreated	3% Ginger	3% Garlic	1.5%Ginger + 1.5%Garlic	0.5%-K-Sorbate	
0	$<10^{1c}$	$<10^{1c}$	$<10^{1c}$	$<10^{1c}$	$<10^{1c}$	**$<10^{1e}$**
2	$4.5x10^{5ab}$	$5.3x10^{5a}$	$4.6x10^{5ab}$	$3.8x10^{5ab}$	$2.8x10^{5abc}$	**$4.2x10^{5a}$**
4	$3.1x10^{5abc}$	$3.9x10^{5ab}$	$3.2x10^{5abc}$	$3.1x10^{5abc}$	$2.5x10^{5abc}$	**$3.1x10^{5ab}$**

6	$2.4x10^{5abc}$	$2.6x10^{5ab}c$	$2.3x10^{5abc}$	$2.5x10^{5abc}$	$2.2x10^{5abc}$	**$2.4x10^{5bc}$**
8	$1.8x10^{5abc}$	$1.9x10^{5abc}$	$1.6x10^{5bc}$	$1.7x10^{5bc}$	$1.5x10^{5bc}$	**$1.7x10^{5cd}$**
10	$1.6x10^{5bc}$	$1.5x10^{5bc}$	$1.1x10^{5ab}$	$1.3x10^{5ab}$	$1.2x10^{5abc}$	**$1.3x10^{5ab}$**
12	$1.8x10^{5abc}$	$<10^{1c}$	$<10^{1c}$	$1.3x10^{5ab}$	$<10^{1c}$	**$1.2x10^{5cde}$**
14	$1.1x10^{5abc}$	$<10^{1c}$	$<10^{1c}$	$<10^{1c}$	$<10^{1c}$	**$4.1x10^{4de}$**
Preservative main effect	**$2.3x10^{5a}$**	**$1.9x10^{5a}$**	**$2.1x10^{5a}$**	**$2.2x10^{5a}$**	**$1.5x10^{5a}$**	

Values are means from replicated determinations: n= 16 for the preservative main effect; n= 10 for the duration of storage main effect; n= 2 for the interactions. Means bearing different superscripts in the duration of storage main effect column, preservative main effect row, and the interaction cells are significantly ($p<0.05$) different.

5.1 CONCLUSION

In conclusion, cold smoked catfish *Clarius gariepinus* can be microbiologically stable and safe for human consumption with minimal changes to the nutrient composition when treated with some natural spices like ginger and garlic under refrigeration storage. Furthermore, the use of up to 3% of these natural spices individually and in combination can compare favourably with synthetic antimicrobial agents like potassium sorbate, citric acid, and sodium metabisulphite. The use of these natural preservatives will therefore help in dousing the tension about health concerns posed by the invasion of the hazardous pathogenic organisms which are associated with the consumption of cold smoked fish under refrigerated storage conditions by reducing and inhibiting the growth of these pathogens in cold smoked catfish. Furthermore, the preservative effect of these spices in cold storage of catfish would be better pronounced after eight (8) days, even showing nearly complete inhibition of these pathogenic organisms. The use of 3% ginger or garlic or a combination of both at an equal 1.5% would therefore enhance the preservation of refrigerated cold smoked catfish.

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