Original Research Article

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

Abstract

Aim: In this study, the shelf stability and microbiological quality of cold-smoked Catfish (*Clarius gariepinus*) at ambient storage were investigated in relation to the preservative effects of potassium sorbate, ginger, garlic, and a combination of ginger and garlic.

Methodology: From the MOUAU Fish Farm, one hundred (100) freshly caught catfish were processed and divided into five lots of twenty each. (We created) Four marinating baths were created, each containing 0.5% potassium sorbate, 3% ginger, 3% garlic, and a mix of 1.5% ginger and 1.5% garlic. All catfish lots, with the exception of the fifth lot (used as a control), were marinated for two hours and cold-smoked for eight hours. Following storage for four days at room temperature (28 2 °C), analyses were performed on the samples. Analyses were (done) conducted on the catfish samples' total viable count, total fungal count, and bacterial counts (*Escherichia coli, Salmonella, Staphylococcus sp, and Listeria sp*).

Result: The total viable count (TVC) was increased from $8.7x10^5$ to $3.43\ x10^6$ CFU/g, $1.13\ x\ 10^6$ to $3.48x10^6$ CFU/g, $1.09\ x\ 10^6$ to $3.28\ x10^6$ CFU/g, $9.6\ x\ 10^5$ to $3.45\ x10^6$ CFU/g, and $1.19\ x\ 10^6$ to $3.66x\ 10^6$ CFU/g for 3% ginger, 3% garlic, 1.5% ginger +1.5% garlic, 0.5% potassium sorbate, and the control at ambient storage conditions respectively. The 1.5% ginger +1.5% garlic was the most effective for *E. coli while* 0.5% potassium sorbate was for *Salmonella* at ambient storage. All the treatments were effective against *Listeria monocytogenes* while the total fungal count increased at ambient storage conditions.

Conclusion: The study (found) revealed 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 4 days at ambient storage provided the storage condition is maintained.

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

1. INTRODUCTION

Fish is desirable for food and business for a number of reasons. It is a major source of animal protein, a significant part of many people's diets, and it provides the essential nutrients for a healthy lifestyle. In comparison to other proteins of animal origin, fish protein now takes precedence and compares favorably with those found in milk, eggs, and meat in terms of amino acid composition [1]. Magawata and Shina [2] reported that “Fish is a very nutrient-dense food that is especially prized for its superior quality protein to that of meat and eggs." Fish is a good source of thiamine, riboflavin, minerals, phosphatides, sterols, enzymes, hormones, hydrocarbons, and pigments. It also contains very high-quality oil and fats [3].

Fish is extremely perishable. Poor or insufficient post-harvest processing and management of fish is one of the biggest challenges facing fish consumption and business in Nigeria, despite how important it is for healthy living and for the generation of income. Fish is processed into smoked or canned fish, as well as other fish-based products, due to this level of perishability.

Smoking is a traditional method of keeping fish fresh in Nigeria. Salt is used as the primary preservative when smoking in the chambers of traditional kilns made of clay, cement blocks, or drums in fishermen's camps. Technically, the length of smoking determines the moisture content of fish products. The moisture (or water activity) of smoked fish has been found to be a key factor in determining the rate of microbial growth and invariably the shelf life or keeping qualities of the fish [5]. Gram [6] notes that samples of crabmeat and smoked fish have both contained microbes like Listeria monocytogenes. Furthermore, according to Da Silva [7], these microbes are common in nature and are capable of growing at both low temperatures and high salt concentrations of up to 10%. Therefore, reducing the fish's water activity to a point where microorganisms can no longer grow has an impact on preservation by drying [8]. Additionally, using salt as a preservative is linked to a number of health issues, necessitating the use of alternative and natural spices like ginger, garlic, etc.

Cold smoking is not widespread in Nigeria, in contrast to the country's traditional method of smoking fish. The smoke's temperature in this method doesn't go above 30°C [8]. In contrast to hot-smoked and oven-dried tilapia samples, Oyelese [9] found that cold-smoked samples had the highest moisture content. This is the reason why cold-smoked fish does not last very long because it has not completely dried out. Studies, such as Eyo [8], have compared cold- and hot-smoked fish, finding that the former has higher nutritional value than the latter because it was cooked during the smoking process.

Finding a substitute for salt as a preservative in fish smoking and extending the shelf life of cold-smoked fish (have) has become more and more important. These preservatives, garlic and ginger, have a variety of biologically active compounds with therapeutic properties [10,11]. Therefore, this study evaluates the

shelf stability and microbiological quality of cold-smoked catfish at ambient temperature using 3% ginger, 3% garlic, a blend of 1.5% ginger and 1.5% garlic, and 0.5% potassium sorbate as preservatives.

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 2hrs 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 ambient temperature and wrapped with foil for 4 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,) making 3 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 the 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 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 instructions. 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 a 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 mould 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.

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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 at ambient temperature at (28± 2^{0}C) for four (4) days. Samples were also taken for microbial analysis in alternate days of the storage periods (i.e., day 0, 2, and 4). The specific microorganisms that were evaluated in the cold smoked fish samples during the storage period exhibited their defined colony characteristics on the respective chromogenic 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 Room (Ambient) Temperature

It was observed that the initial total viable count of all the cold smoked fish before the ambient storage day 0 was low, with the lowest number of count obtained in the 3% ginger treated sample ($8.7x\ 10^5$ cfu/g) and the highest number found in the untreated sample ($1.19x10^6$ cfu/g). 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 load present in the fish samples though statistically, there was no significant difference ($p<0.05$) between the treated and the untreated samples. However, as the duration of storage increased, there was significant increase in the number of total viable count in all the sample but 3% garlic recorded the least increase ($1.13x10^6$ – $2.84x10^6$– $3.48\ x10^6$ cfu/g) and the highest in the untreated sample ($1.19x10^6$ – $3.38\ x10^6$ –$3.66x10^6$ cfu/g). However, the treated samples were significantly different ($p<0.05$) from the untreated sample on day 2 but not significantly different ($p>0.05$) on day 4. This report concurred with the findings of Mosarrat *et al*. [15] who recorded an increase in TVC in salt + garlic-treated smoke-dried *Mastacembelus pancxalus* (biam) during different storage conditions. The effect of preservatives on the TVC from the preservative main effect shows that the treated samples are significantly different ($p<0.05$) from the untreated sample.

Table 1: Effect of Preservatives and Cold Smoking on Total Viable Count TVC (CFU/g) of Catfish stored at ambient temperature (28± 2^0C)

	Duration of storage (Days)			
Preservative	0	2	4	**Preservative main effect**
Untreated	$1.19x10^{6c}$	$3.38x10^{6a}$	$3.66x10^{6a}$	**$2.74x10^{6a}$**
3% Ginger	$8.7x10^{5c}$	$2.43x10^{6b}$	$3.43x10^{6a}$	**$2.33x10^{6b}$**
3% Garlic	$1.13x10^{6c}$	$2.84x10^{6b}$	$3.48x10^{6a}$	**$2.48x10^{6b}$**
1.5%Ginger+1.5%Garlic	$1.09x10^{6c}$	$2.66x10^{6b}$	$3.28x10^{6a}$	**$2.34x10^{6b}$**
0.5% K-Sorbate	$9.6x10^{5c}$	$2.87x10^{6b}$	$3.45x10^{6a}$	**$2.27x10^{6b}$**
Duration of storage main effect	**$9.5x10^{5c}$**	**$2.89x10^{6b}$**	**$3.46x10^{6a}$**	

Values are means from replicated determinations: n= 6 for preservative main effect; n= 10 for duration of storage main effect; n= 2 for the interactions. Means bearing different superscripts in the preservative main effect column, duration of storage main effect row and the interaction cells are significantly ($p<0.05$) different.

This implies that the preservatives used have significant effect on the TVC compared to the untreated sample. The effect of storage on TVC from duration of storage main effect shows that TVC of cold smoked fish samples were increased with increased in the duration of ambient storage due to the growth and multiplication of microbes. This agrees with the results of Bilgin *et al*. [16] and Kester *et al*. [17], who reported an increase, in TVC of Gilthead Seabream (*Sparus aurata*) after cold and hot smoking and an increase in microbial load of cold smoked Atlantic Cod *Gadus morhua* during ambient storage respectively. Furthermore, there was significant difference ($p<0.05$) between the treated and the untreated sample, which means that though there was an increase, the rate of the increase was slow in the treated samples compared to the untreated sample. This increase could be a result of the absorption of a small amount of moisture by the processed fish from the surrounding atmosphere which provides an enabling environment for microbial growth as a result of the increased duration of storage at ambient temperature.

3.2 Effect of Preservatives and Cold Smoking on Total Fungal Count (CFU/g) for Catfish stored at Room (Ambient) Temperature.

At the beginning of the storage period day 0, it was observed as shown in Table 2 below that 3% ginger ($4.6x10^5$ CFU/g) treated sample recorded the lowest value of the total fungal count (CFU/g) and 3% garlic (6.1x105 CFU/g) recorded the highest count. However, there was no significant difference ($p> 0.05$) among the samples. But as the duration of storage increased, the total fungal count significantly increased ($p<0.05$) in all the samples, with 3% ginger recording the lowest ($6.6x10^5$– $7.4x10^5$ CFU/g) and the 0.5% potassium sorbate ($7.1x10^5$ - $8.2x10^5$ CFU/g) the highest TFC among the treated samples throughout the storage days, although there was no significant difference ($p>0.05$) among the treated samples. It was noted that there was a general increase among the samples but, the total fungal count of the untreated sample was significantly higher than the treated samples as the duration of storage increased. This agrees with Kester *et al*. [17], who reported an increase in mould count of cold–smoked Atlantic cod under ambient storage. This could be attributed to the anti-fungal effect of the preservatives.

Statistically, the effect of preservatives from preservative main effects on the total fungal count shows, significant difference ($p<0.05$) between the treated and the untreated samples. 3% ginger ($6.2x10^5$ CFU/g) treated sample was most effective, followed by 3% garlic ($6.5 x10^5$ CFU/g), 1.5 % ginger + garlic ($6.9 x10^5$ CFU/g), 0.5% potassium sorbate then untreated sample ($7.7 x10^5$ CFU/g) which had the highest value of total fungal count.

This result shows that these preservatives have anti-fungal properties and it agrees with Negbenebor *et al*. [20] who used clove and ginger individually and in combination to reduce the fungal load of smoked fish. The effect of storage on the TFC from the duration of storage main effect shows a clear significant difference ($p<0.05$) in the storage days 0, 2 and day 4, ($5.6x10^5$, $7.0x10^5$ and $7.3x10^5$ CFU/g) respectively.

Table 2: Effect of Preservatives and Cold Smoking on Total Fungal Count (CFU/g) of Catfish stored at ambient temperature (28± 2^0C)

	Duration of storage (Days)			Preservative
Preservative	0	2	4	**main effect**
Untreated	$5.7x10^{5de}$	$8.2x10^{5ab}$	$9.1x10^{5a}$	**$7.7x10^{5a}$**
3% Ginger	$4.6x10^{5e}$	$6.6x10^{5bcd}$	$7.4x10^{5abcd}$	**$6.2x10^{5b}$**
3% Garlic	$6.1x10^{5cde}$	$6.4x10^{5bcde}$	$7.1x10^{5bcd}$	**$6.5x10^{5b}$**
1.5%Ginger+1.5%Garlic	$5.8x10^{5de}$	$6.9x10^{5bcd}$	$7.9x10^{5abc}$	**$6.9x10^{5ab}$**
0.5% K-Sorbate	$5.7x10^{5de}$	$7.1x10^{5bcd}$	$8.2x10^{5ab}$	**$7.0x10^{5ab}$**
Duration of storage main effect	**$5.6x10^{5c}$**	**$7.0x10^{5b}$**	**$7.3x10^{5a}$**	

Values are means from replicated determinations: n= 6 for preservative main effect; n= 10 for duration of storage main effect; n= 2 for the interactions. Means bearing different superscripts in the preservative main effect column, duration of storage main effect row and the interaction cells are significantly ($p<0.05$) different.

This is indicative that storage has significant effect on total fungal count which implies that TFC increased with increase in the duration of storage of cold smoked samples. This work is in agreement with Idris *et al*. [23] and Kester *et al*. [17], who reported an increase in TFC of different concentrations of ginger-treated catfish under storage and an increase in mould count of cold smoked Atlantic cod, Gadus morhua at ambient storage respectively.

3.3 Effect of Preservatives and Cold Smoking on *Escherichia* coli Count (CFU/g) for Catfish stored at Room (Ambient) Temperature.

It was observed from the result that there was no detectable growth of *Escherichia coli* in all the samples at day 0. However, as the duration of storage increased at day 2, the growth of *E. coli* was noticed with the sample treated with 1.5% ginger + 1.5% garlic ($3.0x10^5$ CFU/g) recording the lowest number of growth and 0.5% potassium sorbate ($6.4x10^5$ CFU/g) the highest number of growth among the treated samples. Values obtained from the untreated samples was significantly higher ($p<0.05$) than those of the treated samples, probably because of the antimicrobial effect of the preservatives applied to the fish prior to cold smoking. Further increase in the number of *E. coli* was also observed as the duration of storage increased but without any significant difference ($p<0.05$) between the treated and the untreated samples.

Table 3: Effect of Preservatives and Cold Smoking on *Escherichia coli count* (CFU/g) of Catfish stored at ambient temperature (28± 2^0C)

	Duration of storage (Days)			Preservative main effect
Preservative	0	2	4	
Untreated	ND	$9.2x10^{5a}$	$6.2x10^{5c}$	**$5.1x10^{5a}$**
3% Ginger	ND	$4.0x10^{5ef}$	$5.5x105^{cd}$	**$3.2x10^{5b}$**
3% Garlic	ND	$4.5x10^{5de}$	$5.5x105^{cd}$	**$3.3x10^{5b}$**
1.5%Ginger+1.5%Garlic	ND	$3.0x10^{5f}$	$4.2x10^{5e}$	**$2.4x10^{5c}$**
0.5% K-Sorbate	ND	$6.4x10^{5c}$	$7.7x10^{5b}$	**$4.7x10^{5a}$**
Duration of storage main effect	**$<10^{1b}$**	**$5.4x10^{5a}$**	**$5.8x10^{5a}$**	

Values are means from replicated determinations: n= 6 for preservative main effect; n= 10 for duration of storage main effect; n= 2 for the interactions. Means bearing different superscripts in the preservative main effect column, duration of storage main effect row and the interaction cells are significantly ($p<0.05$) different. ND = Not Dictated

The effect of preservative on *E. coli* from the preservative main effect shows significant difference ($p<0.05$) among the treated samples. Sample treated with 1.5% ginger + 1.5% garlic ($2.4x10^5$ CFU/g) was seen to be most effective against *E. coli*, followed by 3% ginger ($3.2x10^5$ CFU/g), and 3% garlic ($3.3x10^5$ CFU/g) then 0.5% potassium sorbate ($4.7x10^5$ CFU/g) which was the least effective. However, the growth rate of *E. coli* on the treated samples, were significantly lower than the untreated sample. This means that

the preservatives used were effective against *E. coli*. This is in agreement with Ezeama and Ozua [21] who stated that ginger and garlic possess antimicrobial properties.

The effect of storage on *E. coli* from the duration of storage main effect revealed that, between the days 2 and 4 storage ($5.4x10^5$ and $5.8x10^5$ CFU/g) respectively, there was no significant difference ($p<0.05$) between them which means that storage significantly had equal increase / effect in the growth rate of *E coli* on both storage days hence the longer the duration of storage the higher the growth rate of *E coli*, but are significantly different ($p<0.05$) from day 0 ($<10^1$ CFU/g) which had no detectable growth of *E coli* in all the samples. This disagrees with Kester *et al*. [17] who found no detectable amount of *E coli* throughout the storage days of cold smoked Atlantic Cod *Gadus morhua*.

3.4 Effect of Preservatives and Cold Smoking on *Staphylococcus aureus* Count (CFU/g) for Catfish stored at Room (Ambient) Temperature.

From the values obtained in Table 4, it was observed that at day 0, sample treated with 3% ginger had no detectable growth of *Staphylococcus aureus* ($<10^1$CFU/g), this was followed by 0.5% potassium sorbate ($2.8x10^5$ CFU/g) ,1.5% ginger + 1.5% garlic treated sample ($3.1x10^5$ CFU/g) and the 3% garlic treated sample, which are not significantly different from each other, then the untreated sample ($4.4x10^5$ CFU/g) which recorded the highest number of growth.

Table 4: Effect of Preservatives and Cold Smoking on *Staphylococcus aureus count* (CFU/g) of Catfish stored at ambient temperature (28± 2^0C)

Preservative	Duration of storage (Days) 0	2	4	Preservative main effect
Untreated	$4.4x10^{5fg}$	$6.3x10^{5b}$	$9.3x10^{5a}$	**$7.0x10^{5a}$**
3% Ginger	$<10^{1h}$	$5.9x10^{5d}$	$7.5x10^{5bc}$	**$4.4x10^{5d}$**
3% Garlic	$3.5x10^{5ef}$	$4.5x10^{5e}$	$7.0x10^{5c}$	**$5.0x10^{5c}$**
1.5%Ginger+1.5%Garlic	$3.1x10^{5g}$	$8.3x10^{5b}$	$8.0x10^{5bc}$	**$6.5x105^{a}$**
0.5% K-Sorbate	$2.8x10^{5g}$	$7.3x10^{5bc}$	$8.1x10^{5b}$	**$6.1x10^{5b}$**
Duration of storage main effect	**$2.8x10^{5c}$**	**$6.6x10^{5b}$**	**$8.0x10^{5a}$**	

Values are means from replicated determinations: n= 6 for preservative main effect; n= 10 for duration of storage main effect; n= 2 for the interactions. Means bearing different superscripts in the preservative

main effect column, duration of storage main effect row and the interaction cells are significantly ($p<0.05$) different.

Furthermore, with increase in the duration of storage the number of *S. aureus count* in the samples increased with significant difference ($p<0.05$) among the samples. This agrees with Kester *et al*. [17] who recorded an increase in *S. aureus* count in cold smoked Atlantic cod.

However, the result obtained from the preservative main effect show significant difference ($p<0.05$) among the treated samples. 3% ginger had the most effect ($4.4x10^5$ CFU/g) against *S. aureus*, followed by 3% garlic ($5.0x10^5$ CFU/g) ,0.5% potassium sorbate ($6.1x10^5$ CFU/g), and 1.5% ginger + garlic ($6.5x10^5$ CFU/g) which had the least effect on *S. aureus* . This means that the different preservatives used had varying effect

on the fish samples but values obtained are significantly lower than the untreated samples ($7.0x10^5$ CFU/g). The effect of storage on the *S. aureus* from the duration of storage main effect shows significant difference ($p<0.05$) in the storage days ($2.8x10^5$ CFU/g), ($6.6x10^5$ CFU/g), and ($8.0x10^5$ CFU/g) for days 0, 2 and 4 respectively. This implies that storage has significant effect on the *S. aureus* and that *S. aureus* count increased with increase in the duration of storage.

3.5. Effect of Preservatives and Cold Smoking on *Listeria sp* Count (CFU/g) for Catfish stored at Room (Ambient) Temperature.

From the result obtained in Table 5. *Listeria* sp was not detected in all the samples at days 0 ($<10^1$ CFU/g) and day 2 ($<10^1$ CFU/g) storage days. However, with increase in the duration of storage, ($3.9x10^5$ CFU/g) from 3% ginger, ($3.8x10^5$ CFU/g) from 3% garlic, ($3.0x10^5$ CFU/g), from 1.5% ginger + garlic, ($3.1x10^5$ CFU/g) from 0.5% potassium sorbate and ($4.8x10^5$ CFU/g) from the untreated sample were isolated from the samples. This result could be attributed to the inhibitory effect of the phenolic compounds in the wood and the antimicrobial effect of the preservatives applied on the treated samples against *Listeria spp.*

Table 5: Effect of Preservatives and Cold Smoking on *Listeria* sp *count* (CFU/g) of Catfish stored at ambient temperature (28± 2^0C)

Preservative	Duration of storage (Days) 0	2	4	Preservative main effect
Untreated	$<10^{1d}$	$<10^{1d}$	$4.8x10^{5a}$	**$1.6x10^{5a}$**
3% Ginger	$<10^{1d}$	$<10^{1d}$	$3.9x10^{5b}$	**$1.3x10^{5ab}$**
3% Garlic	$<10^{1d}$	$<10^{1d}$	$3.8x10^{5bc}$	**$1.3x10^{5ab}$**
1.5%Ginger+1.5%Garlic	$<10^{1d}$	$<10^{1d}$	$3.0x10^{5c}$	**$9.8x10^{4b}$**
0.5% K-Sorbate	$<10^{1d}$	$<10^{1d}$	$3.1x10^{5bc}$	**$1.0x10^{5b}$**
Duration of storage main effect	**$<10^{1b}$**	**$<10^{1b}$**	**$3.7x10^{5a}$**	

Values are means from replicated determinations: n= 6 for preservative main effect; n= 10 for duration of storage main effect; n= 2 for the interactions. Means bearing different superscripts in the preservative main effect column, duration of storage main effect row and the interaction cells are significantly ($p<0.05$) different.

Also, the emergence of this organism at day 4 during the ambient storage may likely be due to the low temperature inherent in cold smoking process which provides a favourable condition for proliferation of *Listeria* sp*,* if the catfish harboured the pathogen or acquired it from the processing environment prior to cold smoking or storage environment during storage. Moreover, there were significant differences ($p<0.05$) among the samples, the treated samples recorded lower counts of *Listeria* sp than the untreated sample, and the anti-listeria activity of the preservatives could have accounted for this.

The effect of preservatives from preservative main effect on *Listeria* sp shows no significant difference ($p>0.05$) among the treated samples. However, the 1.5% ginger+1.5% garlic ($9.8x10^4$ CFU/g) treated sample had the lowest count and hence most effective against the organism, followed by 0.5% Potassium sorbate ($1.0x10^5$ CFU/g) then 3% ginger and 3% garlic ($1.3x10^5$ and$1.3x10^5$ CFU/g) respectively. Moreover, values obtained from the treated samples were significantly lower and different ($p<0.05$) from the untreated sample ($1.6x10^5$ CFU/g). This result shows the *anti listeral* properties of the different preservatives used in this study. Hence the study agrees with Neetoo *et al.* [22] who reported antilisterial activity of some preservatives potassium sorbate combined with Nisin on cold smoked salmon pata and fillet. Therefore, these indicated that the preservatives are effective and inhibitory to *Listeria* sp when compared to the untreated sample.

The effect of storage on this pathogen from the duration of storage main effect shows no significant difference ($p>0.05$) between day 0 and day 2 but they are significantly different from day 4 the last day of ambient storage. This is an indication that ambient storage significantly affected *Listeria* sp from 4th day of storage by increasing the counts across the samples. Therefore, *Listeria* sp counts was increased with increase in duration of storage at ambient temperature of cold smoked spice treated catfish.

3.6 Effect of Preservatives and Cold Smoking on *Salmonella* spp Count (CFU/g) for Catfish stored at Room (Ambient) Temperature.

It was observed from the table below that there was no detectable count of *Salmonella* sp in all the samples at day 0 as shown in Table 6. However, as the duration of storage was increasing the organism became visible across the samples. At day 2 storage, 0 .5 % potassium sorbate treated sample recorded the least count ($6.4x10^5$ CFU/g), while 3% ginger ($9.0x10^5$ CFU/g CFU/g) the highest count among the treated samples, there were significant variation in the rate of increase among the samples. Further increase was also observed as the duration of storage increased to day 4. Also, a decrease ($9.4x10^5$ - $8.2x10^5$ CFU/g) was observed in the 3% garlic at day 4. (Please input references to support your statement)

The effect of preservatives from the preservative main effect indicated a significant variation among the samples. 0.5% potassium sorbate ($4.5x10^5$ CFU/g) was most effective in inhibiting the organism followed by 1.5% ginger + garlic ($5.1x10^5$ CFU/g) then 3% garlic and 3% ginger ($5.9x10^5$ and $6.1x10^5$ CFU/g) respectively. Moreover, 0.5% potassium sorbate and 1.5% ginger + 1.5% garlic treated sample were significantly lower than the untreated sample. This result implies that these treatments given to the fish samples had significant effect on *Salmonella*, but 3% garlic and 3% ginger were significantly higher than the untreated sample.(Please input references to support your statement)

The effect of storage from the duration of storage main effect statistically on *Salmonella* shows no significant different ($p>0.05$) at day 2 and day 4 ($7.9x10^5$ and $8.3x10^5$ CFU/g) respectively but they are significantly different from day 0 ($<10^1$ CFU/g). This means that storage had no effect on the organism at

day 0 but with increase in the duration of storage there was a significant increase ($p<0.05$) in *Salmonella* count in all the samples. (Please input references to support your statement)

Table 6: Effect of Preservatives and Cold Smoking on *Salmonella spp* (CFU/g) of Catfish stored at ambient temperature (28± 2^0C)

	Duration of storage (Days)			Preservative main effect
Preservative	0	2	4	
Untreated	$<10^{1f}$	$7.6x10^{5cd}$	$8.7x10^{5abc}$	**$5.4x10^{5bc}$**
3% Ginger	$<10^{1f}$	$9.0x10^{5ab}$	$9.3x10^{5ab}$	**$6.1 x10^{5ab}$**
3% Garlic	$<10^{1f}$	$9.4x10^{5a}$	$8.2x10^{5bc}$	**$5.9x10^{5ab}$**
1.5%Ginger+1.5%Garlic	$<10^{1f}$	$7.1x10^{5de}$	$8.3x10^{5abc}$	**$5.1x10^{5c}$**
0.5% K-Sorbate	$<10^{1f}$	$6.4x10^{5e}$	$7.1x10^{5de}$	**$4.5x10^{5d}$**
Duration of storage main effect	**$<10^{1b}$**	**$7.9x10^{5a}$**	**$8.3x10^{5a}$**	

Values are means from replicated determinations: n= 6 for the preservative main effect; n= 10 the for duration of storage main effect; n= 2 for the interactions. Means bearing different superscripts in the preservative main effect column, duration of storage 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 ambient 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 storage conditions by reducing and inhibiting the growth of these pathogens in cold smoked catfish. However, the use of these natural preservatives at ambient storage, however, may not offer the needed preservative effect beyond four days.

(All numberings highlighted in red should be removed)

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