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Effect of Ginger and Garlic Marinates on Growth of Microbial Contaminants in Cold-Smoked Catfish (*Clarius gariepinus)* Stored at Room Condition

Ubadire-Agua C. [a], Ezeama C. F. [a], Anyiam C. A. [b,c*], Nwanagba N. L. [a] and Anyiam C. C. [d]

[a] Department of Food Science and Technology, Micheal Okpara University of Agriculture, Umudike, Nigeria.
[b] Department of Food Technology, Akanu Ibiam Federal Polytechnic, Unwana, Nigeria.
[c] Department of Biology, Morgan State University, United States.
[d] Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors U-AC and ECF designed the study, literature searches, wrote the protocol, and performed the analyses of the study. Author ACA wrote the first draft of the manuscript. Authors NNL and ACC managed the statistical analysis of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: In this study, the preservative effect of potassium sorbate, ginger, garlic and a combination of ginger – garlic on the microbiological quality and shelf stability of cold-smoked Catfish (*Clarius gariepinus*) were investigated.

**Corresponding author: Email: anyiamchukwudum@gmail.com, Anyiamchukwudum17@gmail.com;*

Methodology: A total of 100 fresh catfish from Michael Okpara University of Agriculture, Umudike (MOUAU) Nigeria Fish Farm was used for the analysis. The fish were processed and divided into five lots of twenty each. This was followed by marinating in baths containing 0.5% potassium sorbate, 3% ginger, 3% garlic, and a mix of 1.5% ginger and 1.5% garlic each and a control for 2h. Afterwards, the treated fish was cold-smoked for 8 h and then stored at room temperature (28 ± 2°C). Fish samples were collected at 2-day intervals to determine total viable count, total fungal count, and bacterial counts (*Escherichia coli, Salmonella, Staphylococcus* sp*, and Listeria* sp.).
Results: Findings of this study revealed that potassium sorbate, ginger, garlic, and ginger-garlic blends enhanced the microbiological safety of cold–smoked Catfish (*Clarius gariepius).* The result showed that although counts of some microbial contaminants increased in the cold-smoked fish, however, it varied ($p<0.05$) with the treatment during storage. Comparatively, 1.5% ginger - 1.5% garlic marinate was the most effective and performed considerably higher ($p<0.05$) than 0.5% potassium sorbate in the reduction of the microbial contaminants. In this study, the lowest total viable counts were observed in the 3% ginger–treated cold smoked fish ($8.7x10^5$ cfu/g) while the untreated sample was the most contaminated (1.19×10^6 cfu/g) at pre-storage. A significant increase in the total viable count was observed in all the samples during storage however, 3% garlic recorded the least (1.13×10^6 to 2.84×10^6 to 3.48×10^6 cfu/g) and the highest in the untreated sample (1.19×10^6 to 3.38×10^6 to 3.66×10^6 cfu/g). Overall, 1.5%ginger+1.5%garlic blend was most effective on *E. coli* while 0.5% potassium sorbate was on *Salmonella* at ambient storage. *Listeria monocytogenes* was most susceptible to all the treatment, the growth of fungi steadily increased in the treated cold smoked fish during ambient storage.
Conclusion: The study found that 3% ginger, 3% garlic, 1.5% ginger - 1.5% garlic mixture inhibits significantly the growth of microbial contaminants relatively with 0.5% potassium sorbate in cold-smoked fish during ambient storage.

Keywords: Microbial quality; ambient condition; ginger; garlic; potassium sorbate; bacterial count.

1. INTRODUCTION

Fish is desirable for food as a source of animal protein, to provide the essential nutrients for a healthy lifestyle. Consequently, fish protein takes precedence over 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 perishable, poor or insufficient post-harvest processing and its management is one of the biggest challenges facing its consumption. Fish is processed into smoked or canned fish, as well as other fish-based products, due to this level of perishability [4].

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 is a factor in determining microbial growth and shelf life of fish [5]. Gram [6] notes that crabmeat and smoked fish contained Listeria monocytogenes. Accordingly, Da Silva [7], have reported that although 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 associated with several health issues. For this reason the use of alternative and natural spices like ginger, garlic are necessary.

Cold smoking is not widespread in Nigeria to the traditional method of smoking fish. In cold smoking method, the temperature doesn't go above 30°C [8]. In contrast to hot-smoked and oven-dried tilapia samples, Oyelese [9] reported that cold-smoked fish have higher moisture content due to improper drying. As a result, the cold – smoked fish has a shorter shelf stability. Besides, study by Eyo [8], revealed that the cold smoked fish has higher nutritional value than the hot – smoked fish. Finding a substitute for salt as a preservative in fish smoking and extending the shelf life of cold-smoked fish have become more

necessary 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

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± 2^0C) 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.

2.1 Sample Collection

One hundred (100) samples of Catfish (*Clarias gariepinus*) with weights ranging between 300 to 400g were purchased from the Michael Okpara University of Agriculture, Umudike's fish farm Nigeria. The ginger, garlic, and other marinating ingredients were purchased from Ubani main market, Abia State Nigeria. The fish samples were transported to the Laboratory Department of Food Science and Technology Michael Okpara University of Agriculture, Umudike Nigeria prior to analyses

2.2 Processing 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.

2.3 Fish Processing and Smoking

The Catfish was processed by cutting open through the head down to remove the guts and gills, It was then washed with running tap water, and placed in five (5) lots of 20 pieces each. Applying Marinating standards by World Health Organization 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- 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.

2.4 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.

2.5 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.

2.6 Microbial Analysis

The enumeration of contaminants of the fish were determined according to standard

procedure described by Ezeama [15]. A 25 g of the fish was homogenised in 225 ml sterile distilled water. Aseptically, the resultant stock solution was tenfold serially diluted and pour plated on 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) . The inoculated agar Petri plates were then incubated at 37^{o}C for 24 h except Dichloran Bose-Bengan Chloramphenicol (DRBC) Oxoid for yeast and mould. Discrete colonies formed ; Pink colonies were observed in the plate and was counted using colony county indicating the presence of *Escherichia coli* /Coliforms. Green colonies were observed and counted with colony counter which indicated the presence of *Listeria* spp. purple and blue colonies were counted and recorded with a colony counter, indicating the presence of *Salmonella* spp. Black colonies with halo around it was observed and counted using colony counter indicating the presence of *Staphylococcus* spp. and the presence of yeast and mould were enumerated and expressed as colony forming unit per gram of samples (cfu/g).

2.7 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. RESULTS AND DISCUSSION

3.1 Effect of Preservatives and Cold Smoking on Total Viable Count (CFU/g) for Catfish Stored at Room (Ambient) Temperature

Table 1. presents the effect of preservatives and cold smoking on total viable count (cfu/g) for catfish stored at room (ambient) temperature. In this study, lowest total viable count was observed in the 3% ginger-treated cold smoked fish (8.7x 10^5 cfu/g), while the untreated sample was the most contaminated (1.19x10^6 cfu/g) at pre-storage. 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 a significant increase in the number of total viable counts in all the samples. However, 3% garlic recorded the least increase (1.13x10^6 – 2.84 x10^6 – 3.48 x10^6 cfu/g). The untreated sample (1.19x10^6 – 3.38 x10^6 –3.66x10^6 cfu/g) was most contaminated. 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. [16] 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^{0}C)

Preservative	Duration of storage (Days)			Preservative main effect
	0	2	4	
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. [17] and Kester et al. [18], 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. [18], 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. [19] 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 main effect
Preservative	0	2	4	
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: Means with different superscripts along the column, and row varied significantly at p<0.05

This is indicative that storage has a significant effect on total fungal count which implies that TFC increased with an increase in the duration of storage of cold smoked samples. This work is in agreement with Idris et al. [20] and Kester *et al*. [18], 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 growths among the treated samples. Values obtained from the untreated samples were 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.

The effect of preservatives on *E. coli* from the preservative main effect shows a significant difference ($p<0.05$) among the treated samples. The 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* in the treated samples was significantly lower than in 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 ($p<0.05$) 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. [18] 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 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
Preservative	0	2	4	**main effect**
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

Table 4. Effect of preservatives and cold smoking on *Staphylococcus aureus count* (CFU/g) of catfish stored at ambient temperature (28± 2^{0}C)

Preservative	Duration of storage (Days)			Preservative main effect
	0	2	4	
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. [18] 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^{0}C)

Preservative	Duration of storage (Days)			Preservative main effect
	0	2	4	
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 the 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 preservative 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 were increased with an 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 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.

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.

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
Preservative	0	2	4	**main effect**
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

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.

4. CONCLUSION

In conclusion, cold smoked catfish *Clarius gariepinus* can be microbiologically stable and safe for human consumption 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. However, the use of these natural preservatives at ambient storage, however, may not offer the needed preservative effect beyond four days.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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