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COMBINED ANTI-BACTERIAL EFFECTS OF TURMERIC (*Curcuma longa*) AND BLACK PEPPER (*Piper guineense*) ON THE SHELF LIFE OF SMOKED *Clarias gariepinus* (BURCHELL, 1822)

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Abstract

The effects of combined anti-bacterial effects of turmeric and black pepper on the shelf life of smoked *C. gariepinus* were evaluated. The fresh turmeric rhizome and dried black pepper fruits were ground into fine powder. **A combined powder** was formulated by mixing homogenously a 1:1 turmeric-black pepper powder mixture tagged **TBP**. The TBP was prepared by formulating 0%, 10%, 20% and 30% powder concentrations. The experimental fish samples were then assigned to the four experimental treatments in triplicates and were soaked for one hour then smoked for 16 hours at 85^0c ± 5^oc. The smoked treated catfish fish samples were stored for eight weeks and analyzed bi-weekly for pH and microbial loads. Results for pH assessment showed the control treatment values decreased from 6.033±0.03 after 24hrs to 4.573±0.401 at week 8. For the 30%TBP, the values decreased from 6.187±0.015 after 24hrs to 6.120±0.010 at week 8. All treatments pH values were within acceptable limit (5.5-6.2) except for the control's pH at week 8 (4.573±0.40). Difference in pH between treated samples and control was significant ($p<0.05$). At week 8, the highest and lowest bacterial Total Plate Count load of $8.7x10^6$ and $3.0x10^4$CFU/g was recorded in the control and the 30TBP respectively, and the difference in bacterial load between treated samples and control was significant ($P<0.05$). The 30TBP treatment had the lowest final Lactic Acid Bacterial Count (LABC) value ($4.1x10^2$±0.08 CFU/g), while the highest LABC ($2.4x10^7$±0.03^d CFU/g) was in the control and the difference in bacter ial load between treated samples and control was significant ($P<0.05$). The 30TBP treatment had the lowest final Fungal Count value ($7.8x10^3$±0.03^a CFU/g), while the highest Fungal Count ($8.3x10^6$±0.02^c CFU/g) was in the control and the difference in fungal load between treated samples and control was significant ($P<0.05$). This result showed that the Combined Turmeric and Black Pepper powder (TBP) treated smoked *C. gariepinus* maintained a stable acceptable pH and mitigated microbial growth as compared to the control. Therefore, it is recommended that, in order to improve the quality and shelf life of preserved smoked *C. gariepinus*, the 30%TBP spice treatment can be used.

Key Words: *antibacterial, pH, shelf life, smoked fish.*

1.0 Introduction

Fish is one of the most perishable of all foods and a medium for growth of microorganisms. At ambient temperature, spoilage is rapid (Ndimele *et al.*, 2019). Fish are highly perishable food at ambient temperature and high quantity of fish spoils due to lack of good preservation methods (Jadhav and Anal, 2018). Consequently, preservation is required unless it is consumed soon after capture; because of its susceptibility to spoilage (Ahmad *et al.*, 2021 in Olatunde *et al*., 2022). Fish spoilage can be caused by 3 main mechanisms: enzymatic degradation, oxidative rancidity of fat and microbial spoilage (Nath *et al.,* 2021). Fish invariably becomes putrid within a few hours of capture unless they are subjected to some form of processing or preservation. Various food products have different spoilage times that are referred to as shelf-life (Humaid and Jamal, 2014). Therefore, preservation techniques are needed to prevent fish spoilage and extend their shelf life, inhibit the activity of spoilage bacteria and the metabolic changes that result in the loss of fish quality (Ndimele *et al.*. 2019).

Farid *et al.* (2015) reported that, the problem associated with the smoke-drying process is the infestation by insects followed by microbial spoilage of fish. Recent advances to preserve fishes with antibiotics to reduce the chances of microbial spoilage are although effectively successful, but the development of antibiotic-resistant bacterial strains is of great concern and difficult to control (Nath *et al.,* 2021). Synthetic additives and preservatives with antimicrobial and antioxidant properties play an important role in ensuring food safety. Many antimicrobial agents such as sulfites, nitrites, organic acids and several antioxidants (such as butylated hydroxyl anosole, butylated hydroxytoluene, butylhydroxyquinone) have been used since long to preserve fish from microbial spoilage and oxidative rancidity. Despite the high potential for preservation, some restrictions are there in the application of these synthetic preservatives due to potential toxicological effects (Viji *et al.*, 2015).

There is need to improve the traditional fish smoking method in order to prolong the shelf life of smoked fish. One strategy that can help to achieve this is the use of antioxidants (Ndimele *et al.*, 2019).

Spices are edible plants materials that possess antioxidative, antiseptic and bacteriostatic properties (Ndimele *et al.*, 2019). The antimicrobial and preservative activities of conventional spices on fish products have been known for some time and these have been exploited in the preservation of smoked-dried fish.

Turmeric and black pepper possesses excellent antimicrobial, immunostimulatory, antioxidant, antifungal properties and flavor enhancing attribute, thus increasing its potential to be used in fish preservation, providing low cost and nutritive fish and fishery products with extended shelf life and minimal toxicity (Nath *et al.,* 2021).

Several authors have reported various levels of success with the use of spices (garlic,

ginger, turmeric, and others) on shelf life or organoleptic properties enhancement of fish. None of the study has been reported for extension of the shelf life of smoked *Clarias gariepinus* in ambient storage using combination of turmeric and black pepper.

Curcumin from the spice turmeric, exhibit anti-inflammatory, antioxidant, anti-cancer, antiviral and neurotrophic activity and therefore hold promise as a therapeutic agent to prevent and treat several disorders. However, a major barrier to curcumin clinical efficacy is its poor bioavailability (Lopresti, 2018). However, piperine, the active ingredient in black pepper, can help to make curcumin more bioavailable**.** With just 1/20 teaspoon or more of black pepper, the bioavailability of turmeric is greatly improved, and turmeric's benefits are further enhanced (UMass Chan Medical School, 2019). Thus, the present study is to investigate the application of turmeric in combination with black pepper powder to enhance the shelf life and quality attributes of *Clarias gariepinus* ambient during storage.

2.0 Materials and methods

Samples Collection

Thirty (30) life *Clarias gariepinus* fish species with average weight of 500g ±5g was purchased at Old Bukuru park fish market, Jos, Plateau state, Nigeria, and then transported for smoking at the smoking kiln installed beside the Hydrobiology and Fisheries Laboratory, University of Jos, Jos, Plateau state, Nigeria. The spices, fresh turmeric (*Curcuma longa*) rhizome and dried black pepper (*Piper guineense*) seeds was purchased at terminus market, Jos, Plateau state, Nigeria.

Preparation of *Clarias gariepinus* Samples

The fresh water *C. gariepinus* were washed in salt water for disinfection and removal of external dirt, and then was eviscerated. Each piece was aseptically cut into two equal pieces with some skin incisions to allow for increased surface area for absorption of spices. They were then washed again with clean water, and were allowed to drain for 30 minutes.

Preparation of Turmeric and Black Pepper Powder

The fresh turmeric rhizome and dried black pepper fruits were properly washed and rinsed with distilled water, air-dried and ground into fine powder using electric blender and then tagged *Curcuma longa* powder (**CLP**) and *Piper guineense* powder (**PGP**) respectively. **A third combined powder** was formulated by mixing homogenously 50% CLP and 50% PGP to make a homogenous mixture of turmeric powder and black pepper powder tagged **TBP**. The combined turmeric/black pepper powder (TBP) was prepared by formulating 0g (control), 10g, 20g, and 30g in 100ml of water to form 0% (control treatment), 10%, 20% and 30% powder concentration according to the method of Adeyemi *et al.* 2013 in Edward, 2021.

Preparation of Experimental Fish Samples for Processing

500 ± 5g of the experimental fish samples were assigned to four experimental treatments. These are the control (with 0g powder), 10g, 20g and 30g powder

concentration. Each of the treatment was replicated in triplicate. The experimental fish was then soaked in the powder paste for 1 hour. Thereafter the fish was properly drained for 30 minutes according to the method of Adeyemi et al. 2013 in Edward, 2021.

Fish Smoking and Storage

Fire was set in the smoking kiln with selected firewood (the African birch, *Anageissus leiocarpus)* as heat source, until the trays in the kiln are heated enough that hands cannot be laid upon for 5 seconds. The fish was set in the firewood smoking kiln subjected to hot smoking until a constant weight is achieved. The smoke from the kiln was produced by the burning of the firewood. Uniform heat distribution and drying was ensured by exchanging the trays. The fish was smoked for 16 hours at 85± 5^{o}C. This was followed by sun drying to make fish muscle compressed and to prevent breaking of smoked products. Smoked products were packed in sealed transparent sterile polyethylene bags to reduce microbial contamination as described by Salán et al., (2006). Each packet consists of body and tail part of the fish. The polyethylene bags were later perforated after an initial sample for analyses was taken to allow for free flow of ambient air. Each treatment of the smoked fish were arranged, labelled and stored in air-free wire-netted cage to prevent flies and rodents contamination and to enhance flow-through ventilation throughout the storage period. The netted cage was placed on laboratory shelves at ambient temperature (29±3^{o}C) for eight weeks. The fish samples were subjected to physico-chemical, and microbiological analyses bi-weekly during the eight weeks storage period (Adeyemi *et al.*, 2013; Ndimele *et al*., 2019).

Determination of pH

Ten grammes (10g) of blended fish samples was placed in a container with 100ml of distilled water and stirred. The pH was read using Multifunction Water Quality Tester (set to pH and Temperature) (Bolourin and Khodaparast, 2010; Saeed, 2009).

Total Plate Count (TPC)

Ten (10) grams of fish sample was aseptically transferred to a stomacher bag and 90ml of sterile Salt Peptone Solution (SPS) as diluents was added (20g peptone/1000ml distilled water). The sample was homogenized in a stomacher (Seward 400 Stomacher Lab Blender /Stock 36001) for 90 seconds to obtain a stock solution. A serial dilution (10^{-1} to 10^{-5}) of the homogenized samples was made using sterile distilled water. 100µLof the last two dilutions was inoculated on Plate Count Agar (PCA) using the spread plate method. Plates were incubated at 37^{o}C for 18-24 hours. Viable count was calculated as colony forming unit CFU/g sample (ICMSF, 2011).

Fungal Count (FC)

Ten (10) grams of fish sample was aseptically transferred to a stomacher bag and 90ml of sterile Salt Peptone Solution (SPS) as diluents was added (20g peptone/1000ml distilled water). The sample was homogenized in a stomacher (Seward 400 Stomacher Lab Blender /Stock 36001)

for 90 seconds to obtain a stock solution. A serial dilution (10^{-1} to 10^{-5}) of the homogenized samples was made using sterile distilled water. 100µLof the last two dilutions was inoculated on Potato Dextrose Agar (PDA) using the spread plate method. Plates were incubated at ambient temperature for 24- 48 hours. Viable count was calculated as colony forming unit CFU/g sample (ICMSF, 2011).

Lactic Acid Bacteria Count (LABC)

Ten (10) grams of fish sample was aseptically transferred to a stomacher bag and 90ml of sterile Salt Peptone Solution (SPS) as diluents was added (20g peptone/1000ml distilled water). The sample was homogenized in a stomacher (Seward 400 Stomacher Lab Blender /Stock 36001) for 90 seconds to obtain a stock solution. Serial dilutions (10^{-1} to 10^{-5}) of the homogenized samples were made using sterile distilled water. 100µL of the last two dilutions was inoculated on de Man Rogosa Sharpes (MRS) agar using the spread plate method. Plates were incubated at 37ºC for 24 - 48 hours. Viable count was calculated as colony forming unit CFU/g sample (ICMSF, 2011).

3.0 Data Analysis

All the findings were expressed as mean (n=3). Analysis of variance (ANOVA) was applied to the data obtained, and T-test for comparing initial and final means of data using GraphPad Prism, version 8.2. Statistical significance was set at $p< 0.05$. Fisher's Least Significant Difference was used to separate differences in treatment means.

4.0 Results

Effects of Combined Turmeric/Black Pepper Powder (TBP) on pH values of smoked *Clarias gariepinus*

The pH values obtained during the 8 weeks storage period of smoked *Clarias gariepinus* spiced with combined turmeric/black pepper paste (TBP) are as shown on Figure 1. The pH values decrease with increase in storage time. For the control, the values decreased from 6.033±0.03 after 24hrs to 4.573±0.401 at week 8. For the 30%TBP, the values decreased from 6.187±0.015 after 24hrs to 6.120±0.010 at week 8. Although the pH values shows a general decrease with increasing storage time, at week 8, there was no significant difference ($p>0.05$) between treated samples. However, difference between treated samples and control was significant ($p<0.05$). **Acceptable pH of hot-smoked storage fish is a pH of 5.5- 6.5 (**Eyo, 2001; Kin-Kabari *et al*., 2011; ICMSF, 2011)**.** Therefore, we can infer that the pH values of the combined turmeric/black pepper paste treated samples were all within the acceptable range; however, for the control, at week 8 the pH (4.573±0.40) decreased below the acceptable range of 5.5-6.2.

Effects of Combined Turmeric/Black Pepper Paste (TBP) on Total Plate Count (TPC) of Smoked *Clarias gariepinus*

Result of mean total plate count (CFU/g) of smoked *Clarias gariepinus* spiced with combined turmeric/black pepper paste (TBP) is shown in Table 1. The lowest and highest initial bacterial load of $1.2x10^{3}$±0.02 and $5.4x10^{4}$±0.01 CFU/g was in the 30TBP and control respectively; however, differences in

initial bacterial load among the treatments was not significant (P>0.05). At week 8, the highest and lowest bacterial load of $8.7x10^6 \pm 0.02$ and $3.0x10^4 \pm 0.05$ was recorded in the control and the 30TBP respectively, and the difference in bacterial load between treated samples and control was significant (P<0.05). The microbial load values recorded were within the safe limit ($<10^5$ -$<10^7$ CFU/g) as reported by FAO and WHO (2013). Generally, the bacterial total plate count recorded, increased with increase in storage period and decreased with increase in spice concentration.

Combined Turmeric/Black Pepper Paste (TBP) Effect on Lactic Acid Bacterial Count (LABC) of Smoked C*larias gariepinus*

Result of combined turmeric/black pepper paste (TBP) effect on lactic acid bacterial count (LABC) of smoked Clarias *gariepinus* is shown in Table 2. The lowest initial LABC ($1.7x10^2 \pm 0.06$) was in the 30TBP while the highest LABC ($2.4x10^7 \pm 0.03$) at week 8 was in the control. Generally, LABC of combined turmeric/black pepper treated samples was lower than that of the control. There was a steady increase in LABC values with storage period in all cases and decreased with increase in TBP concentration. At week 8, although the 30TBP treatment had the lowest LABC value ($4.1x10^2 \pm 0.08$), there was significant difference (P<0.05) between all the treatments. Combined turmeric/black pepper treated samples LABC values was within the acceptable range of $<10^5$ – $<10^7$CFU/g (FAO &WHO, 2013 in Olatunde et al. 2022).

C**ombined Turmeric/Black Pepper Paste (TBP) Effect on Fungal Count of Smoked *Clarias gariepinus***

Result of Mean Fungal count (CFU/g) of smoked *Clarias gariepinus* spiced with combined turmeric/black pepper paste (TBP) for eight weeks storage period is shown in Table 3. The lowest initial fungal count value ($8.6x10^2 \pm 0.03$) was in the 30TBP while the highest fungal count ($8.3x10^6 \pm 0.02$) at week 8 was in the control. Generally, fungal count of combined turmeric/black pepper treated samples was significantly lower (P<0.05) than that of the control. There was a steady increase in fungal count with storage period in all cases and decreased with increase in spice concentration. At week 8, the 30TBP recorded the lowest fungal count ($7.8x10^3 \pm 0.03$). Difference between treated samples and the control was significant (P<0.05).

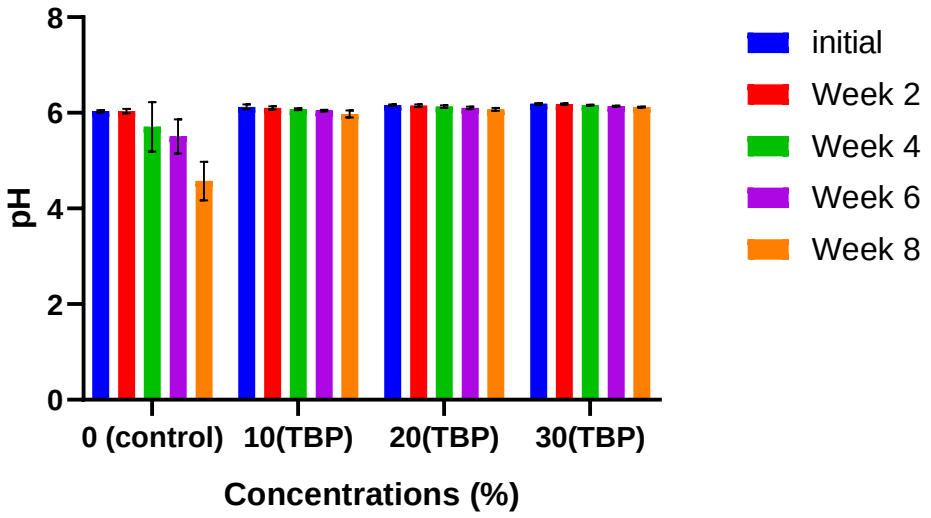

Figure 1: Mean pH Values of Smoked *Clarias gariepinus* Spiced with Combined Turmeric/Black Pepper Paste (TBP) for Eight Weeks Storage Period (Temperature Range: 27.8± 2^{o}C)

Table 1: Mean Total Plate Count (CFU/g) of Smoked *Clarias gariepinus* Spiced with Combined Turmeric/Black Pepper (TBP) for Eight Weeks Storage Period

Conc. (%)	**After 24hrs**	**Week 2**	**Week 4**	**Week 6**	**Week 8**
0 (control)	$5.4x10^{4}\pm0.01^{a}$	$6.2x10^{5}\pm0.03^{b}$	$6.4x10^{5}\pm0.02^{b}$	$7.6x10^{6}\pm0.03^{c}$	$8.7x10^{6}\pm0.02^{d}$
10(TBP)	$4.0x10^{3}\pm0.00^{a}$	$4.0x10^{3}\pm0.04^{a}$	$4.6x10^{3}\pm0.01^{a}$	$5.5x10^{4}\pm0.02^{b}$	$6.2x10^{4}\pm0.05^{c}$
20(TBP)	$3.8x10^{3}\pm0.01^{a}$	$3.9x10^{3}\pm0.03^{a}$	$4.1x10^{3}\pm0.03^{a}$	$4.7x10^{4}\pm0.03^{b}$	$5.1x10^{4}\pm0.04^{b}$
30(TBP)	$1.2x10^{3}\pm0.02^{a}$	$3.6x10^{3}\pm0.03^{a}$	$3.8x10^{3}\pm0.05^{a}$	$2.9x10^{4}\pm0.02^{a}$	$3.0x10^{4}\pm0.05^{a}$

All values are means of triplicate readings, ± standard deviations of means. Values with different superscripts in the same row/column are significantly different (P<0.05).

Table 2: Mean Lactic Acid Bacterial Count (LABC) of Smoked *Clarias gariepinus* Spiced with Combined Turmeric/Black Pepper Paste (TBP) for Eight Weeks Storage Period

Conc, (%)	After 24hrs	Week 2	Week 4	Week 6	Week 8
0 (control)	$6.1x10^2 \pm 0.01^a$	$2.3x10^3 \pm 0.03^b$	$3.6x10^3 \pm 0.02^b$	$6.9x10^5 \pm 0.03^c$	$2.4x10^7 \pm 0.03^d$
10(TBP)	$3.2x10^2 \pm 0.02^a$	$3.4x10^2 \pm 0.04^a$	$3.9x10^2 \pm 0.03^a$	$4.1x10^2 \pm 0.02^b$	$2.8x10^3 \pm 0.03^c$
20(TBP)	$3.2x10^2 \pm 0.06^a$	$3.4x10^2 \pm 0.02^a$	$3.9x10^2 \pm 0.06^a$	$4.1x10^2 \pm 0.05^b$	$2.8x10^3 \pm 0.11^b$
30(TBP)	$1.7x10^2 \pm 0.06^a$	$2.0x10^2 \pm 0.05^a$	$2.6x10^2 \pm 0.04^a$	$2.9x10^2 \pm 0.07^a$	$4.1x10^2 \pm 0.08^a$

All values are means of triplicate readings, ± standard deviations of means. Values with different superscripts in the same row/column are significantly different (P<0.05).

Table 3: Mean Fungal Count (CFU/g) of Smoked *Clarias gariepinus* Spiced with Combined Turmeric/Black Pepper Paste (TBP) For Eight Weeks Storage Period

Conc. (%)	After 24hrs	Week 2	Week4	Week 6	Week 8
0(control)	$3.4x10^3 \pm 0.01^a$	$6.4x10^3 \pm 0.03^a$	$6.8x10^3 \pm 0.02^a$	$1.7x10^6 \pm 0.03^b$	$8.3x10^6 \pm 0.02^c$
10(TBP)	$3.0x10^3 \pm 0.00^a$	$3.0x10^3 \pm 0.04^a$	$3.8x10^3 \pm 0.01^a$	$4.1x10^4 \pm 0.02^b$	$1.7x10^5 \pm 0.05^b$
20(TBP)	$2.8x10^3 \pm 0.01^a$	$2.9x10^3 \pm 0.03^a$	$3.1x10^3 \pm 0.03^a$	$3.4x10^3 \pm 0.04^a$	$2.2x10^4 \pm 0.02^{ab}$
30(TBP)	$8.6x10^2 \pm 0.03^a$	$7.6x10^2 \pm 0.04^a$	$2.5x10^3 \pm 0.06^a$	$2.7x10^3 \pm 0.01^a$	$7.8x10^3 \pm 0.03^a$

All values are means of triplicate readings, ± standard deviations of means. Values with different superscripts in the same row/column are significantly different (P<0.05).

5.0 Discussion

Effects of Combined Turmeric/Black Pepper Powder (TBP) on pH Values of Smoked *Clarias gariepinus*

The pH values obtained during the 8 weeks storage period were as shown on figure 1. The pH values decrease with increase in storage time. The pH of a fish in storage can decrease with time due to breakdown of protein and lipids, glycolysis, microbial growth, autolytic changes and storage conditions (Zhao *et al*., 2022; Bao *et al*., 2023). At week 8, there was no significance difference ($p>0.05$) between treated samples, however, differences between treated samples and control was significant ($p<0.05$). Acceptable pH of hot-smoked storage fish is a pH of 5.5- 6.5 **(**Eyo, 2001; Kin-Kabari *et al*., 2011; ICMSF, 2011**).** Therefore, we can infer that the pH values of the turmeric and black pepper powder treated samples were all within the acceptable ranges; except for the control at week 8 that decreased beyond the acceptable range of 5.5. A clear indication that Combined Turmeric/Black Pepper Powder (TBP has the ability of keeping the pH of hot smoked storage Clarias stable and hence, extend it shelf life.

This result agrees with the study conducted by Olusola (2021) using onion bulb, holy basil and turmeric rhizome as preservatives on smoked Clarias; Anthonia Da Silva (2002); Alsaiqali *et al*., (2016) and Adeyemi *et al.,* (2013); who also reported a decrease in the pH of smoked *C. gariepinus* as the week of storage increased. This result disagrees with work conducted by Jadhav and Anal (2018) using Nile tilapia meat during ice storage and Ozyurt *et al.,* (2009) for red mullet and goat fish during ice storage. They reported increase in pH due to volatile compounds produced from bacterial spoilage and the addition of alkaline compounds produced from enzymatic degradation.

Effects of Combined Turmeric/Black Pepper Powder (TBP) on Microbial Loads of Smoked *Clarias gariepinus*

Result of mean microbial status (including total plate count, lactic acid bacterial count, and fungal count (CFU/g)) of hot smoked spiced *C. gariepinus* and *O. niloticus* is shown in Tables 1, 2 and 3. Generally, the microbial counts increased with increase in storage period and decreased with increase in spice concentration. At week 8, the highest and lowest microbial loads was recorded in the control and the 30TBP respectively, and the difference in bacterial load between treated samples and control was highly significant ($P<0.05$). Over all, the 30% TBP had the lowest microbial loads as compared to the 10 and 20% spices concentration. This difference might be due to the increase in spice concentration.

The microbial loads of treated samples recorded were within the safe limit ($<10^5$ - $<10^7$ CFU/g) as reported by FAO and WHO, 2013, and Olatunde *et al*., 2022). This observation is similar with that of Olatunde et al (2022) who reported an increasing trend in both Total Viable Count (TVC) and Mould Count (MC) within two samples and between the spiced and unspiced samples with increased storage period. Isaac et al.(2014) using mixture of salt and pepper to preserve smoked Clarias, observed that bacterial total viable count values decreased as the concentration of salt and pepper inclusion

increased and treated groups recorded decrease in microbial loads compared to the control. Gupta and Ravishankar, (2005) demonstrating the antimicrobial activity of ginger, garlic and turmeric on Escherichia coli also supports this claim. Ihuahi et al. (2007) determining the preservative effect of brine, pepper and garlic spice mixture on hot-smoked *C. gariepinus* stated that there was a steady increase in mould count with storage period, and that spiced-treated samples showed lower count.

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