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Effects of spring onion (*Allium fistulosum*) and turmeric (*Curcuma longa*) marinade on the shelf life of smoke-dried *Clarias gariepinus*

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ABSTRACT

This study was conducted to determine the effect of spring onion marinade and turmeric marinade on the microbial load of smoked-dried *Clarias gariepinus* stored at ambient temperature (37^0C) for six weeks. Forty (40) fishes with an average weight of 500g were gutted, washed and randomly assigned to the treatments. The experimental treatments were the control, turmeric, spring onion and turmeric. The fishes were soaked in the treatments for 20 minutes. After smoke-drying, the fishes were stored and comparative analysis of the microbial load of each treated fish samples were done biweekly for six (6) weeks. The fish samples were analysed using Potato Dextrose Agar and Nutrient Agar for fungi and bacteria respectively. There was a general increase in microbial load as the storage period progressed. After 6 weeks of storage at ambient temperature, the study showed sample B (Spring Onion) had the highest (1.3×10^6 cfu/g) mean bacterial load and the least ($8.8x10^5$ cfu/g) was in sample C. Samples A (control) and C (turmeric marinade) had the highest (1.4×10^5 cfu/g) mean fungal load while the least was seen in Sample B (9.3×10 cfu/g). Seven bacterial species namely, *Bacillus subtilis, Enterobactera erogenes, Pseudomononas sp., Streptococcus pyogenes, Micrococcus sp., Proteus sp., and Klebsiella sp* and six fungal species namely, *Mucor piriformis, Candida sp., Aspergillus sp, Rhizopus sp., Penicillium sp, and Fusarium sp* were observed in the study. It is recommended that turmeric marinade should be employed in smoke-drying fish as it had the least microbial load and as such it would have the longest shelf life.

Keywords: Ambient temperature; microbial load; fungal species

INTRODUCTION

Essential fatty acids, minerals, vitamins, and animal protein may all be found in abundance in fish. Its amino acid content makes it a very suitable food for humans, outperforming meat, eggs, and milk in terms of nutritional value (Feldhusen, 2000). Because it contains little collagen, it is a very easily digested meal. It makes up about 40% of the total amount of animal protein consumed, making it a significant part of the typical Nigerian diet. When compared to other food sources of protein, fish is a more affordable primary source of dietary protein (Fawole *et al.*, 2007). When compared to beef, poultry, and mutton, fish muscle is less rough and more digestible because it includes four fundamental elements in

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varied proportions: water (approximately 70–80%), protein (about 16-25%), lipids (about 1-5%), Minerals (1-2%) and vitamins (> 1%) (Kumolu-johnson *et al.,* 2015). It is also a great source of lipids, lysine, iodine, and vitamins A, B, and E (Lund, 2013).

It is imperative to state the significance of fish in the developing world, where it provides many people with both food and income. Fish protein is now more widely available to the teeming population in Nigeria because of the growth of the aquaculture industries brought about by different government development initiatives that promote private sector engagement. Over six million people are employed directly or indirectly in Nigeria's fishing industry, which is expected to contribute roughly 3.55% of the country's GDP (Trade Invest Nigeria, 2010).

Fish is a very perishable item, hence processing surplus will be necessary to reduce post-harvest losses with increased fish output (Bechtel, 2003). According to Doyle (2007), fish spoilage is a change in the food's nutritional and sensory qualities that renders it unpalatable to customers. Fish supply in Nigeria is significantly impacted by fish spoilage stability. Because of insufficient preservation methods and faulty storage, Nigeria loses around 40% of its yearly catch. Temperature, handling techniques, and initial microbial load all affect fish quality. Diverse techniques for preserving fish, including smoking, frying, drying, salting, freezing, chilling, marinating, and canning, have been employed to preserve fish stocks and maintain seafood quality (Bluwey *et al.,* 2018).

Fish processing also involves the use of preservatives, which prolong the fish's shelf life by inhibiting or delaying the growth of microorganisms that lead to spoilage. They are materials that are either artificial or natural (Seetaramaa *et al.,* 2011). Synthetic preservatives have been found to be helpful in reducing rancidity; however, due to their unfavorable effects on liver and lung enzymes, they are prohibited in many countries and are consequently difficult to get (Ikeme and Bhandary, 2001). Because natural preservatives like herbs and spices have little to no negative impact on consumers' health, their use has become necessary (Krishnan *et al.,* 2014).

Fish smoking is the most often used technique in Nigeria. It is a preservation technique that combines drying with the natural chemical deposits that come from the heat degradation of wood (Ahmed *et al.,* 2010). According to Kumolu-Johnson and Ndimele (2011), the procedure has been demonstrated to be useful for extending shelf life, improving taste, and boosting protein availability all year round. Fish that has been smoked is used more often because the natural wood-smoke chemicals, such as phenols, aldehydes, benzoates, and tars, are deposited on the surface of the fish, creating a protective layer that inhibits the growth of other microorganisms and helps preserve fish by drying, cooking, and acting as an effective antioxidant, bacteriostatic, and bactericidal agent (Daramola *et al.,* 2013). Depending on the sort of product required and the species of fish utilized, different nations have different methods for smoking fish (Nahid *et al.,* 2016).

Allium fistulosum, or spring onions, are a rich source of minerals (potassium, copper, chromium, manganese, iron), dietary fibers, folate, vitamin A, C, B2, K, thiamine, calcium, and flavonoids like quercetin, which have anti-inflammatory and antihistamine properties (Crystal *et al.,* 2003). Spring onions' high chromium concentration lowers the risk of diabetes via enhancing glucose tolerance and blood sugar regulation. Additionally, antibacterial qualities aid in the defence against the flu, the common cold, and the alleviation of gastrointestinal aches and pains (Singh & Ramakrishna, 2017).

Asiatic in origin, turmeric (*Curcuma longa*) is a highly appreciated spice that finds extensive application in biological systems as a preservative, antimicrobial, antiviral,

anticancer, and wound healing agent (Gul and Bakht, 2015). Cancer, bacterial infections, excessive cholesterol, premenstrual syndromes, rheumatism, osteoarthritis, skin disorders, diabetes, stomach ulcers, and many other illnesses are among the many ailments for which it is beneficial (Chandran and Goel, 2012; Rivera-Mancia *et al.,* 2015; Lang *et al.,* 2015; Fanaei *et al.,*2015). More than 300 naturally occurring substances, including beta-carotene, ascorbic acid, calcium, flavonoids, fiber, iron, niacin, potassium, zinc, and other minerals, are found in turmeric, which also boosts the body's antioxidant capacity (Li *et al.,* 2011).

MATERIALS AND METHODS

Collection of Materials

Forty (40) newly harvested African catfish (*Clarias gariepinus*) samples of the same size, weight, and stock were acquired from Uteh farm settlement in Benin City, Edo state, Nigeria. To avoid contamination, clean tap water was used to collect fish samples for the study, which were then packed in clean jute bags. Ten (10) fish samples were included in each of the four (4) batches (A, B, C, and D) from which the fish were separated. Batch B was treated with spring onions, Batch C was treated with turmeric, and Batch D was a mixture of both spring onions and turmeric powder. Batch A served as the control group, receiving no treatment. The study utilized plant components that were procured at Uselu market in Benin, namely spring onions (*Allium fistulosum*) and turmeric powder (*Curcuma longa*).

Preparation of Plant Materials

After gathering the spring onions, contaminants were eliminated and thoroughly cleaned. It was then air dried for three to five hours, and then oven dried at 45^0C. The plant material was dried, and then a milling machine was used to grind it into powder. The herb known as turmeric was purchased as powder. The experiment employed marinade spring onions and turmeric.

Preparation of Fish Samples

The fish samples were gutted, properly cleaned, and then submerged in a 15% brine solution (water and common salt). The bowl weighed 0.4 kilogram, the control weighed 4.1 kg, the spring onion weighed 3.8 kg, the turmeric weighed 4.6 kg, and the combination of the spring onion and turmeric weighed 4.8 kg. They were all weighed using an electric weighing scale (Camry measuring scale of 20 kg capacity).

In this study, 2% of the fish's fresh weight was spice-treated, as described by Shamima *et al.* (2007). The marinade treatments were applied to the fish samples according to the methods of Adeyemi *et al.* (2013). Four (4) distinct plastic bowls were used to prepare the two (2) distinct treatments (marinade). It was a control sample, meaning no treatment was administered. In order to produce the second marinade, 40g of spring onions were mixed with 1000ml of water (4% marinade), resulting in 152g of marinade that was applied to 3.85kg of fish.

For the third treatment, 4.6 kg of fish received 184g of turmeric marinade, which was made by mixing 40g of turmeric powder with 1000ml of water (4% marinade). The marinade (4% marinade) for the fourth treatment, which involved applying 192g of spring onion and

turmeric marinade (96g of each) to 4.8kg of fish, was made by combining 20g of each of the two powders-spring onions and turmeric-with 1000ml of water. Following that, the bowls were labeled with the names of the various treatments: Batch A (control; no treatment), Batch B (spring onion treatment), Batch C (turmeric marinade treatment), and Batch D (spring onion + turmeric marinade treatment). According to Mosarrat *et al.* (2017), they were left in the mixture for twenty minutes and then drained.

The Smoking Process

The Magbon-Alade smoking kiln was used to dry the fish samples that had been spice-treated. To avoid contaminating the drips from other treatments, they were thoughtfully placed in separate containers. Until a steady weight was reached, the smoking process was conducted for around 24 hours at an internal temperature of 80^0C (Adeyeye *et al.*, 2015). The fish samples were examined and rotated periodically during the smoking process to ensure that the heat was distributed evenly and to give the sample a smooth texture and look. In order to minimize burning, overheating was prevented. The fish samples were taken out of the smoking kiln, weighed, and recorded after they had been smoked.

Storage

The fish samples that had been smoke-dried were taken out of the smoking kiln and allowed to cool at room temperature. They were kept in plastic basins and covered with brown paper. The plastic bowls were kept at room temperature after being sealed to prevent the growth of microorganisms and the absorption of moisture from the surrounding air.

Processed Fish Sample Collection and Analyses

To assess the microbial load, fresh and treated smoke-dried *C. gariepinus* were brought to the lab and examined. For a duration of six weeks, the bacterial and fungal load of the kept fish was examined every two weeks.

Microbial Analysis

Roberts (1978) description of the procedure was used for the samples' microbiological examination. Following the manufacturer's directions, nutrient agar and potato dextrose agar were made after bacteria and fungus were isolated for confirmation.

Experimental Design

The two primary components of the experiment were: i) Plant source (spring onions and turmeric); ii) Storage duration (2 weeks, 4 weeks, and 6 weeks); and iii) control.

Thus, a two-plant source (spring onion and turmeric) × three storage durations (2 weeks, 4 weeks, and 6 weeks) factorial in a complete randomized design (CRD) was used for the experiment. The experiment will be replicated thrice.

Statistical Analysis

The means from the biweekly analysis of the bacterial and fungal loads of the treatments under ambient temperature storage (using potato Dextrose and Nutrient agar) were subjected to analysis of variance table (ANOVA) Version 12.1 of the GenStat software was used for data analysis. Every analysis was done three times. The Duncan Multiple Range Test (DMRT) was used in triplicate for all analyses, and $P < 0.05$ was used to examine mean differences.

RESULTS

Results of the Initial Microbial load of fresh and smoke-dried *Clarias gariepinus*

Tables 1 and 2 present the findings of the initial microbial load of fresh and smoke-dried fish samples. The results indicated that sample A (Control 3.9 ± 10^3) had the greatest mean population count of bacteria, while sample B (2.8 ± 10^3) had the lowest mean count. The means for the population of bacteria were not substantially different ($p<0.05$).

Following smoke drying, *Enterobacterial genes, Proteus sp., Pseudomonas sp., Bacillus subtilis, and Klebsiella sp*. were the bacteria isolates that were shared by all samples. Sample B (spring onion) did not contain any *Pseudomonas sp.*

Sample A (Control 12 and 29.27%) had the highest frequency of incidence and proportion of bacteria right after smoking (Table 1). The lowest frequency of bacteria (7 and 17.07%) was found in sample B.

Table 1: Effects of spring onion and turmeric marinade on the mean bacteria load of smoke-dried *C. gariepinus*

Dilution factor	A	B	C	D
10^1	81	68	76	72
10^2	30	17	25	22
10^3	08	06	08	06
Mean count (cfu/g)	3.9×10^{3A}	2.8×10^{3A}	3.8×10^{3A}	3.0×10^{3A}

Table 2: The total bacteria frequency of occurrence and percentage of *C. gariepinus* immediately after smoking

Bacteria isolates	A		B		C		D	
	F	%	F	%	F	%	F	%
Enterobacter aerogenes	2	3.85	1	1.92	2	3.85	2	3.85
Proteus sp.	3	5.77	2	3.85	3	5.77	2	3.85
Pseudomonas sp.	2	3.85	-	-	1	1.92	1	1.92
Bacillus subtilis	3	5.77	3	5.77	3	5.77	3	5.77
Klebsiella sp.	2	3.85	1	1.92	3	5.77	2	3.85
TOTAL	12	29.27	7	17.07	12	29.27	10	24.39

The results of the effect of spring onion and turmeric powders on the fungal load of smoke-dried *C. gariepinus* are shown in Table 3. There was no significant difference

($p<0.05$), the sample A (Control 7.3×10^{2}) had the highest fungal mean population count and the lowest mean count of fungi population was seen in sample B (4.1×10^{2A}).

Fungal isolates common to all samples were *Aspergillus sp.*, *Fusarium sp.*, *Penicillium sp.* and *Rhizopus sp*. *Fusarium sp.* was only absent in sample B. The highest fungi frequency of occurrence and percentage immediately after smoking occurred in sample A (Control 8 and 29.63%). sample B had the lowest bacteria frequency of occurrence (5 and 18.52%).

Table 3: Effect of Spring Onion and Turmeric Marinade on the Fungal Load of Smoke-dried *C. gariepinus*

Dilution factor	A (Control)	B	C	D
10^{1}	30	22	27	25
10^{2}	09	06	07	07
10^{3}	01	Nil	Nil	Nil
Mean count (cfu/g)	7.3×10^{2A}	4.1×10^{2A}	4.9×10^{2A}	4.8×10^{2A}

Table 4: Frequency of Occurrence of Fungal Isolates of *C. gariepinus* Immediately after Smoke-drying

Fungi isolates	A		B		C		D	
	F	%	F	%	F	%	F	%
Aspergillus sp.	3	10	2	6.67	2	6.67	2	6.67
Penicillium sp.	2	6.67	2	6.67	2	6.67	2	6.67
Fusarium sp.	1	3.33	-	-	1	3.33	1	3.33
Rhizopus sp.	2	6.67	1	3.33	2	6.67	2	6.67
TOTAL	8	29.63	5	18.52	7	25.93	7	25.93

Results of Biweekly Changes in the Bacterial Load of Smoke-dried *Clarias gariepinus* during Ambient Temperature Storage

After two (2) weeks of ambient storage**,** it was observed that there was no significant difference ($p<0.05$) among the mean population of the bacteria. The sample A (Control) had the highest (1.3×10^{4}) mean bacteria count and Sample C (8.5×10^{3}) had the lowest mean count (Table 5). After two (2) weeks of ambient storage, bacterial isolates common to all samples were *Enterobacter aerogenes, Proteus sp, Pseudomononas sp, Bacillus subtilis and Klebsiella sp* (Table 7). The highest bacteria frequency of occurrence and percentage after two weeks of storage occurred in sample A (Control 14 and 28%), sample B had the lowest bacteria frequency of occurrence and percentage 11 and 22% (Table 7).

After four (4) weeks of ambient storage, the results showed that means of bacteria population were significantly different. Sample B (9.5×10^{5}) had the highest mean bacteria count while sample C (4.3×10^{5}) had the lowest mean count (Table 5). After four (4) weeks of ambient storage, bacteria isolate common to all samples were *Bacillus subtilis, Enterobacteraerogenes, Pseudomononas sp., Streptococcus pyogenes, Proteus sp., Klebsiella sp.* and *Micrococcus sp*. *Streptococcus pyogenes* was absent in sample C while *Micrococcus sp* was absent in sample A (Control) and sample C. The highest bacteria frequency of occurrence and percentage after four weeks of storage occurred in sample B (21 and 27.63%), sample C had the least bacteria frequency of occurrence 16 and 21.05% (Table 7).

After six (6) weeks of ambient storage, the results showed that means of bacteria population were significantly ($P<0.05$) different. Sample B (1.3×10^{6}) had the highest mean

bacteria count and sample C (8.8×10^5) had the least mean count. After six (6) weeks of ambient storage, bacterial isolates common to all samples were *Bacillus subtilis, Enterobacteraerogenes, Pseudomononas sp., Streptococcus pyogenes, Micrococcus sp., Proteus sp., and Klebsiella sp. Micrococcus sp.* was absent in sample C. The highest bacteria frequency of occurrence and percentage after six weeks of storage occurred in sample A (24 and 26.97%), sample B (21 and 23.60%) had the least bacteria frequency of occurrence.

Table 5: Biweekly changes in mean bacterial load of smoked-dried *C. gariepinus* during ambient Storage

Dilution factor	Sample	IAS	ATWS	AFWS	ASWS
Mean counts (cfu/g)	A	3.9×10^{3A}	1.3×10^{4A}	5.3×10^{5C}	9.6×10^{5F}
	B	2.8×10^{3A}	1.2×10^{4A}	9.2×10^{5EF}	1.3×10^{6H}
	C	3.8×10^{3A}	8.5×10^{3A}	4.3×10^{5B}	8.8×10^{5E}

IAS = Immediately after storage; ATWS = After two weeks of storage; AFWS = After four weeks of storage; ASWS = After six weeks of storage

After two (2) weeks of ambient storage, there was no significant difference ($p<0.05$) among the mean population of the fungi. Sample A (Control 4.7×10^3) had the highest number of mean fungi count and Sample C (3.1×10^3) had the least mean count (Table 6). Fungi isolates common to all samples were *Aspergillussp, Rhiopussp, Fusariumsp, and Penicillium sp.* The highest fungi frequency of occurrence and percentage after two weeks of storage occurred in sample A (Control was 10 and 27.03%) sample B, C and D all had 9 and 24.32% (Table 7).

After four (4) weeks of ambient storage, results show that means of fungi population were significantly different. sample A (Control) and sample C both had the highest mean bacteria count (6.3×10^4) and Sample A (5.2×10^4) with the lowest mean count (Table 6). Fungi isolates common to all samples were *Mucor piriformis,Candida sp.,Aspergillussp, Rhizopus sp., Penicilliumsp, and Fusariumsp,.Candida sp.* was absent in sample A and C while *Mucor piriformis* was absent in sample B. The highest fungi frequency of occurrence and percentage after four weeks of storage occurred in sample A (Control 18 and 29.51%), sample D had the least frequency of occurrence 13 and 21.31%.

After six (6) weeks of ambient storage, results show that means of fungi population were significantly different. Sample A (Control) and sample C both had the highest fungi mean count (1.4×10^5) and Sample B (9.3×10^4) had the least fungi mean count (Table 6). Fungi isolates common to samples were *Mucor piriformis,Candida sp.,Aspergillussp, Rhizopus sp., Penicilliumsp, and Fusarium sp.* The highest fungi frequency of occurrence and percentage after six weeks of storage occurred in sample A (Control 22 and 27.85%), sample D had the least fungi frequency mean count 17 and 21.52% (Table 7).

Table 6: Biweekly changes in the fungi load of smoked-dried *C. gariepinus* during ambient storage

Samples	IAS (cfu/g)	ATWS (cfu/g)	AFWS (cfu/g)	ASWS (cfu/g)
A	7.3×10^{2A}	4.7×10^{3A}	6.3×10^{4C}	1.4×10^{5F}
B	4.1×10^{2A}	4.0×10^{3A}	5.2×10^{4B}	9.3×10^{4D}
C	4.9×10^{2A}	3.1×10^{3A}	6.3×10^{4C}	1.4×10^{5F}
D	4.8×10^{2A}	3.6×10^{3A}	5.8×10^{4C}	9.9×10^{4E}

IAS = Immediately after storage; ATWS = After two weeks of storage; AFWS = After four weeks of storage; ASWS = After six weeks of storage

Table 7: Biweekly frequency of occurrence of microorganisms isolated from the stored smoke-dried fish samples

Bacteria isolates	SAMPLE A								SAMPLE B							
	Wk 0		Wk2		Wk 4		Wk 6		Wk 0		Wk 2		Wk 4		Wk 6	
	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%
Enterobacter aerogenes	2	3.85	3	6	4	5.26	4	4.495	1	1.92	3	6	4	5.26	4	4.495
Proteus sp.	3	5.77	3	6	3	3.95	4	4.495	2	3.85	2	4	3	3.95	3	3.37
Pseudomononas sp.	2	3.85	3	6	3	3.95	4	4.495	-	-	1	2	1	1.32	3	3.37
Bacillus subtilis	3	5.77	2	6	4	5.26	4	4.495	3	5.77	3	6	4	5.26	3	3.37
Klebsiella sp.	2	3.85	2	4	4	5.26	4	4.495	1	1.92	2	4	4	5.26	3	3.37
Streptococcus pyogenes	-	-	-	-	2	2.63	3	3.37	-	-	-	-	3	3.95	4	4.495
Micrococcus sp	-	-	-	-	-	-	1	1.124	-	-	-	-	2	2.63	2	2.25
Total	12	29.3	14	28	20	26.32	24	26.97	7	17.07	11	22	21	27.63	22	24.72
Fungi isolates	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%
Aspergillus sp.	3	10	3	8.11	4	6.56	4	5.06	2	6.67	3	8.11	4	6.56	4	5.06
Penicillium sp.	2	6.67	3	8.11	4	6.56	4	5.06	2	6.67	3	8.11	3	4.92	3	3.8
Fusarium sp.	1	3.33	2	5.41	4	6.56	4	5.06	-	-	2	5.4	3	4.92	3	3.8
Rhizopus sp.	2	6.67	2	5.4	3	4.92	3	3.8	1	3.33	1	2.7	2	3.28	3	3.8
Mucor piriformis	-	-	-	-	1	1.64	3	3.8	-	-	-	-	2	3.28	4	5.06
Candida sp.	-	-	-	-	2	3.28	4	5.06	-	-	-	-	-	-	2	2.53
TOTAL	8	29.6	10	27.03	18	29.51	22	27.85	5	18.52	9	24.32	14	22.95	19	24.05

Table 7 *Continued*

Bacteria isolates	SAMPLE C								SAMPLE D							
	Wk 0		Wk 2		Wk 4		Wk 6		Wk 0		Wk 2		Wk 4		Wk 6	
	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%
Enterobacter aerogenes	2	3.85	2	4	3	3.95	4	4.495	2	3.85	3	6	4	5.26	4	4.495
Proteus sp.	3	5.77	3	6	4	5.26	4	4.495	2	3.85	2	4	3	3.95	4	4.495
Pseudomononas sp.	1	1.92	2	4	2	2.63	3	3.37	1	1.92	2	4	2	2.63	4	4.495
Bacillus subtilis	3	5.77	3	6	4	5.26	3	3.37	3	5.77	3	6	4	5.26	4	4.495
Klebsiella sp.	3	5.77	3	6	3	3.95	4	4.495	2	3.85	2	4	3	3.95	3	3.37
Streptococcus pyogenes	-	-	-	-	-	-	2	2.25	-	-	-	-	2	2.63	3	3.37
Micrococcus sp	-	-	-	-	-	-	-	-	-	-	-	-	1	1.32	1	1.124
Total	12	29.27	13	26	16	21.05	21	23.6	10	24.39	12	24	19	25	22	24.72
Fungi isolates	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%
Aspergillus sp.	2	6.67	3	8.11	3	4.92	3	3.8	2	6.67	3	8.11	3	4.92	4	5.06
Penicillium sp.	2	6.67	3	8.11	4	6.56	4	5.06	2	6.67	3	8.11	4	6.56	3	3.8
Fusarium sp.	1	3.33	1	2.7	3	4.92	4	5.06	1	3.33	1	2.7	2	3.28	3	3.8
Rhizopus sp.	2	6.67	2	5.4	4	6.56	4	5.06	2	6.67	2	5.4	2	3.28	3	3.8
Mucor piriformis	-	-	-	-	-	-	2	2.53	-	-	-	-	2	3.28	2	2.53
Candida sp.	-	-	-	-	2	3.28	4	5.06	-	-	-	-	-	-	2	2.53
TOTAL	7	25.93	9	24.32	16	26.23	21	26.58	7	25.93	9	24.3	13	21.31	17	21.52

DISCUSSION

According to the study, sample B had the lowest mean population count of bacteria ($2.8x10^3$) and sample A (control, $3.9x10^3$) had the highest before storage. These outcomes might be the consequence of handling fish samples in an unsanitary manner both during harvesting and transit. Sample B had the lowest mean count of fungus (4.1x102A), whereas sample A had the highest mean count of fungi (Control $7.3x10^2$). This indicates that the number of microorganisms in the fish samples was decreased by the smoke-drying process and the marinade treatment with onion springs and turmeric. Abolagba and Iyeru (1998) reported that improper smoke-drying and unhygienic handling of smoke-dried fish products would result in a very high microbial load, suggest that the handling practices during smoking and the smoke-drying process may be the cause of microorganisms in the treated fish samples.

Following a week of room temperature storage, it was noted that there was no statistically significant variation ($p<0.05$) in the average number of bacteria and fungus. The samples A (Control) rose from $3.9x10^3$ to $1.3x10^4$, B increased from $2.8x10^3$ to $1.2x10^4$, C climbed from $3.8x10^3$ to $8.5x10^3$, and D increased from $3.0x10^3$ to $1.2x10^4$ in terms of the bacteria load.

In contrast to Sample C, which had the lowest mean count, Sample A (control) had the greatest mean count of bacteria, but it also had the highest mean count of fungus when compared to Sample D.

Since the fish samples' muscles were already visibly swollen at this point, there's a chance that the moisture content of the dried, smoked fish sample may rise, which will encourage the growth or actvity of these microorganisms. The findings of Eyo (2001) and Adeyemi *et al*. (2013), who suggested that smoked fish samples could have a comparatively greater water activity level a need for microbial growth corroborate this.

After four weeks of ambient storage, results show that means of bacteria population were significantly different as the results for sample B were significantly different for C and D. In comparison to Sample C, which had the lowest mean count of germs, Sample B had the highest mean count. When compared to Sample B, samples A (Control) and C showed the greatest counts of fungus. The increased amount of moisture content in the fish samples during storage might be the cause of the rise in the population of bacteria. High moisture content environments are ideal for the growth of bacteria. Research has indicated that certain organic preservatives, like spring onions, moringa, turmeric, lemon grass, and bay leaf, have the potential to function as both sanitizers and preservatives. This is due to their discovery of antimicrobial activities against certain food-borne microorganisms that are frequently linked to food spoilage and food-borne illnesses (Bukar *et al.,* 2010).

After six (6) weeks of ambient storage, results show that means of bacteria population were significantly different as the results for sample C was significantly different for B and D. Sample A (Control) and Sample C both had the largest number of fungal count when compared to Sample B with the lowest, whereas Sample B had the highest mean count of bacteria compared to Sample C's lowest mean count. By this time, there were visible indications of degradation brought on by the swelling of the muscles as a result of an increase in moisture build up. This is consistent with the findings of Abbas *et al*. (2009), who emphasized that the water content of the product has a significant impact on the microbiological and chemical stability of fish and fish products throughout processing and storage. Different phenolics are found in different regions of the lemon grass and bay leaf

plants, as well as in uncommon combinations of specific phytochemical substances (Bukar *et al.*, 2010). The quantity and diversity of microorganisms recovered in the treated samples were much lower than in the control and brine-treated samples, which may be attributed to these chemicals. Fish is a low-acid meal that, if not handled carefully and processed quickly after harvesting, can easily encourage the growth of infections, especially bacteria (Haruna, 2003). This helps to explain why, over the course of the investigation, heterotrophic bacteria and fungus were growing in all of the fish samples, but the bacterial load was always larger than the fungal burden.

According to Omoruyi *et al.* (2016), bacteria are present in fish's surroundings and nutrition in large quantities, making it hard to avoid. Six fungal genera were recovered from all of the fish samples utilized in this investigation. The majority of the bacteria found were found on fish processors' skins and in the typical flora of fishponds (Fafioye, 2011). Aspergillus sp., Penicillium sp., Fusarium sp., Mucor piriformis, Candida sp., and Rhizopus sp. were the detected fungus isolates. Seven distinct bacterial taxa were also discovered from the fish samples. These include Enterobacter aerogenes, Micrococcus sp., Bacillus subtilis, Proteus sp., Streptococcus pyogenes, and Pseudomonas sp. *Clarias gariepinus* in the wild had gut microbiota that resembled those of Jimoh *et al.* (2009) and Omoruyi *et al.* (2016). A similar result on the presence of *Pseudomonas sp* in smoked salmon was provided by Salihu *et al.* (2008). Pathogens that can result in nosocomial infections are spared by pseudomonas. It is interesting that the common human pathogen Listeria monocytogenes was not found in the fish samples, in contrast to the results of Ehizibolo *et al.* (2007) and Salihu *et al.* (2008). None of the treated fish samples included *Salmonella sp*, another significant pathogen. This conclusion is consistent with the findings of Nwaiwu *et al.* (2011), who demonstrated the anti-Salmonella, anti-Shigella, and anti-Enterobacter properties of the organic preservative hexane extract. This observation, however, ran counter to the results of Onyia *et al.* (2011), who found that Salmonella sp. was among the bacteria recovered from smoked salmon. The difference between the current research and the one by Onyia *et al.* (2011) may be ascribed to the treatment's (spring onion and turmeric marinade) effectiveness as well as the correct maintenance of aseptic procedures throughout the trials. The findings of Bukar *et al.* (2010), who also observed comparable microbiological outcomes as a result of the activities of organic preservatives, support this conclusion. It has been noted that the fungus found in the fish samples are a common source of contamination for smoked fish (Abolagba *et al.*, 2011; Wogu and Iyayi, 2011).

CONCLUSION

The study's findings demonstrated that the microbial population and dynamics of the fish samples were significantly impacted by smoke-drying with powdered spring onions and turmeric. All three smoked-dried fish sample treatments (B spring onion, C turmeric, and D spring onion + turmeric marinade) had a relatively low bacterial and fungal count below the 5 x 105cfu/g recommended by the International Commission of Microbial Specification for Food and Food Products (ICMS, 2002), indicating that the preservatives decreased the growth level of microorganisms Additionally, sample B had a higher bacterial load than samples C (turmeric) and D (spring onion + turmeric marinade), with sample C having the lowest bacterial load. Additionally, it revealed that sample D (spring onion + turmeric marinade) and sample B (spring onion) had the lowest fungal loads, whereas sample C had the highest.

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