Original Research Article

EFFECT OF NATURAL PRESERVATIVES (LEMON GRASS AND BAY LEAF MARINADE) ON THE MICROBIAL LOAD AND SHELF LIFE OF SMOKE-DRIED *Clarias gariepinus*

Abstract

This study was carried out to determine the effect of lemon grass marinade (sample A), bay leaf marinade (sample B) and a combination of both spices (sample C) on the microbial load of stored smoked-dried *Clarias gariepinus*. Comparative analysis of the microbial load of each treated fish samples during ambient storage was done biweekly for six (6) weeks and then samples of each treatment were taken to the laboratory periodically. The fish samples were analysed using Potato Dextrose Agar and Nutrient Agar for fungi and bacteria respectively. The initial microbial load before storage showed that the highest number of bacteria counts occurred in smoke-dried *Clarias gariepinus* treated with bay leaf marinade (4.7×10^3) while the lowest was in smoke-dried *C. gariepinus* treated with lemon grass marinade (1.3×10^3). The highest fungi count was observed in smoke-dried *C. gariepinus* treated with the combination of both spices (9.5×10^2) while the least fungi count was in fresh fish sample (1.4×10^3), although the fresh fish sample had the highest bacteria mean count (5.1×10^3) when compared with the treated samples. After 6 weeks of ambient storage, the result of smoke-dried *C. gariepinus* treated with bay leaf marinade was also significantly different from the mean population of smoke-dried *C. gariepinus* treated with lemon grass marinade and smoke-dried *C. gariepinus* treated with the combination of both spices. Smoke-dried *C. gariepinus* treated with bay leaf marinade again had the highest bacteria and fungi mean count of 1.7×10^5 and 2.3×10^4 respectively. Smoke-dried *C. gariepinus* treated with lemon grass marinade and smoke-dried *C. gariepinus* treated with the combination of both spices had similar bacteria mean count of 1.5×10^5 while smoke-dried *C. gariepinus* treated with lemon grass marinade had the lowest fungi mean count of 1.6×10^4. The study revealed that all three smoked-dried fish sample treatment had a relatively low bacterial and fungal count below the 5 x 10^5cfu/g recommended by the International Commission of Microbial Specification for Food and Food Products (ICMS, 2002).

Keywords

natural preservatives, lemon grass, bayleaf marinade, shelf life

Food spoilage is a change in the nutritional and sensory characteristics of food which makes it unacceptable to consumers [1]. Despite the inability of local fish production to meet the corresponding demand, large quantities of locally produced fish are lost to post-harvest losses ranging from bacterial and autolytic spoilage to other factors. These factors cause fish to lose its organoleptic qualities, and become generally unacceptable for human consumption. Thus it becomes imperative to employ various preservation methods such as drying, smoking, freezing, chilling and brining to conserve fish resources. With the ever growing world population and the need to store and transport the food from one place to another where it is needed, food preservation becomes necessary; in order to increase its shelf life and maintain its nutritional quality, texture and flavour. Therefore, good food preservation techniques must prevent microbial spoilage of food without affecting its quality and nutritional value [2]. Fish smoking is one of the traditional methods of preservation of fish in Africa. Smoke curing, as applied to fish, is a method of preservation effected by combination of drying and the

deposition of naturally produced chemicals, resulting from the thermal breakdown of wood [3]. The reasons for fish smoking are varied but in Nigeria, the process has proven relevant to prolonging shelf-life, enhancing flavour, storing for lean season and increasing protein availability of people throughout the year [4].

Generally, natural preservation techniques and methods are often abandoned for the synthetic methods like the application of pesticides which have been discovered to have adverse effects on the health of the final consumers of such fish, such as cancer, lung problems among others [5]. Lemon grass (*Cymbopogon citratus*) is a rich source of citral, which is used in perfumery, pharmaceutical industries, and bioactive compounds (flavonoids and vitamin C). The natural flavonoids are also attracting more and more attention not only due to their antioxidant properties, but also as anti-carcinogenic and anti-inflammatory agents because of their lipid anti-peroxidation effects [6]. Bay laurel (*Laurus nobilis*) is a plant of industrial importance, used in foods, drugs, and cosmetics. The dried leaves and essential oils are used extensively in the food industry for seasoning of meat products, soups and fishes. Chemically it has been found to contain sesquiterpene lactones such as 10-epigazaniolide, Gazaniolide, spirafolide, costunolide, Reynosin, santamarine, flavonoidglycosides, essential oil. It has been reported to possess wound healing, neuroprotective, antioxidant, antiulcerogenic, anticonvulsant, antimutagenic, antiviral, anticholinergic, antibacterial, antifungal activities [7]. [8] reported that due to the introduction of Bay leaf (*Laurus nobilis* L.). There was an increased amount of crude protein and crude lipid in rainbow trout. This study was therefore carried out to determine the effects of lemon grass marinade (*Cymbopogon citratus*) and bay leaf marinade (*Laurus nobilis*) on the shelf life of African catfish (*Clarias gariepinus*).

Materials and Methods

Collection of Fish Samples

A total of thirty-six (36) freshly harvested African catfish *(Clarias gariepinus)* samples of equal size, age and weight from the same stock was obtained from the Department of Aquaculture and Fisheries Management, University of Benin, Benin city, Edo state Nigeria. The fish samples for the study were collected using a plastic bowl with clean tap water sealed with clean jute bags to prevent contamination. The fishes were then divided into three batches, A, B and C with each batch containing a total of twelve (12) fish samples. Batch A was treated with lemon grass, Batch B was treated with bay leaf while Batch C was treated with both lemon grass and bay leaf.

Collection of Plant Materials

The plant materials used for the study were milled lemon grass (*Cymbopogon citratus*) and bay leaf (*Laurus nobilis*). Bay leaf was purchased from Uselu market in Benin while lemon grass was collected from the Senior Staff Quarters of the University of Benin Nigeria.

Preparation of plant materials

Having collected the plant materials, impurities were removed from them and were washed properly. After which, they were air-dried before being oven-dried at 45°c for about 3-5 hours. After drying, the plant materials were ground into powder using milling machine. The lemon grass and bay leaf powder were used for the experiment.

Preparation of fish samples

The fish samples were prepared through a series of procedures which include; gutting, washing and salting. They were weighed using an electric weighing balance with each sample weighing 0.7g. After gutting and washing thoroughly, they were placed in 15% brine solution (common salt and water). According to [9] common salt retards the activities of bacteria, enzymes and chemicals in fish and salting reduces the slime on the surface of the fish, which also inactivates the surface bacteria. Spice treatment of 2% of fresh weight of fish was used in this study as reported by [10]. Marinade was used in applying the treatments to the fish

samples. This is in accordance with the method of [11]. The three (3) different treatments (marinade) were prepared in three (3) separate plastic bowls. For the first treatment, lemon grass marinade was prepared by adding 40g of the powdered lemon grass to1000ml of water, forming a 4% marinade. Bay leaf marinade was also prepared using 40g of bay leaf powder to 1000ml of water (4% marinade) for the second treatment. The third treatment was prepared by adding 20g each of both lemon grass and bay leaf powders(40g in total) to1000ml of water to make the marinade (4% marinade) for the third treatment.

The bowls were then labelled according to the different treatments; Batch A (treated with lemon grass marinade), Batch B (treated with bay leaf marinade) and Batch C (treated with lemon grass and bay leaf marinade). The fish samples were placed in their respective treatments (12 fish samples for each treatment). They were allowed to stay in the mixture for 20 minutes, followed by draining, according to [12]

The smoking process

The fish samples treated with the spices were placed for drying in the Magbon-Alade smoking kiln. After smoking, the fish samples were removed from the smoking kiln, weighed and recorded.

Storage

After smoking, the smoke-dried fish samples were removed from the smoking kiln and allowed to cool at room temperature. They were then wrapped in brown paper and stored in cartons. The carton was sealed inorder to reduce microbial proliferation and moisture absorption from the environment and then stored at room temperature.

Processed fish sample collection and analyses

Fresh and treated smoke-dried *C.gariepinus* were taken to the Laboratory and analyzed to check for microbial load. Bacterial and fungal analyses were carried out on the samples. The stored fish was subjected to bi-weekly analysis of bacterial and fungal load for a period of six weeks.

Microbial Analysis

For the microbial analysis of the samples, the method that was adopted was that described by [13]. Bacteria and fungi were isolated for confirmation, thus Nutrient agar and Potato dextrose agar were then prepared using the manufacturer's instructions.

Experimental Design

The experimental is made up of two main factors, namely;

i) Plant sources (lemon grass and bay leaf) and

ii) Storage time (2weeks, 4weeks and 6weeks)

The experimental design was therefore a two plant sources (bay leaf and lemon grass) × 3 storage times (2, 4 and 6 weeks) factorial in a complete randomized design (CRD). Experimental trials were conducted in triplicate.

Statistical Analysis

Data analysis was done using GenStat software version 12.1. All analysis were carried out in triplicate using Duncan Multiple Range Test (DMRT) where $P < 0.05$ was applied to study the difference between the means.

Result

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

Results of the initial microbial load of fresh and smoke-dried fish samples can be found in tables 1 and 2. Although the results show that the means for bacteria and fungi population were not significantly different ($p<0.5$) but however the fresh fish sample had the highest bacteria mean population count ($5.1\times103\pm46.00$) when compared with the treated samples, A ($1.3\times10^3\pm12.00$), B ($4.7\times10^3\pm33.67$) and C ($3.3\times10^3\pm24.00$). The lowest mean count of fungi population was seen in sample A ($8.8\times10^2\pm7.00$) while the highest was sample B

147 ($2.1\times10^3\pm12.67$) when compared with the fresh fish sample ($1.4\times10^3\pm14.67$) and sample C
148 ($9.5\times10^2\pm8.33$). Bacteria isolates from the fresh fish samples were *Escherichia coli, Enterobacter aerogenes* and *Pseudomononas aruginosa*.
150 Immediately after smoke drying, bacteria isolates from sample A were *Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Pseudomononas aruginosa* and *Micrococcus sp*,
152 sample B were *Bacillus subtilis, Streptococcus lactis, Enterobacter aerogenes, Proteus sp.,*
153 and *Micrococcus sp* and sample C were *Bacillus subtilis, Streptococcus lactis, Enterobacter*
154 *aerogenes, Pseudomononas aruginosa* and *Micrococcus sp*.
155 The total bacteria frequency of occurrence and percentage before storage for fresh fish
156 sample was 11 and 78.56%, sample A was 7 and 58.33%, sample B was 23 and 71.89% and
157 sample C was 15 and 68.18% respectively. Fungi isolates from fresh fish sample were
158 *Aspergillus sp, Penicillium sp* and *Mucor sp*. Fungi isolates from sample A were *Aspergillus*
159 *sp, Penicillium sp, Fusarium sp*, and *Mucor sp*, sample B were *Aspergillus sp, Penicillium sp,*
160 *Fusarium sp, Mucor sp* and *Candida sp* and sample C were also *Aspergillus sp, Penicillium*
161 *sp, Fusarium sp, Mucor sp* and *Candida sp.* The total fungi frequency of occurrence and
162 percentage before storage of the fresh fish sample were 3 and 23.45%, sample A was 5 and
163 41.67%, sample B was 9 and 28.11% and sample C was 7 and 31.82% (Table 7).

164 **Table 1: Effect of treatment on the bacteria load of fresh and smoke-dried *C.***
165 ***gariepinus***

Dilution factor	Fresh	A	B	C
10^{-1}	92	26	67	49
10^{-2}	35	07	23	15
10^{-3}	11	03	11	08
Mean count	**$5.1\times10^3\pm46.00$a**	**$1.3\times10^3\pm12.00$a**	**$4.7\times10^3\pm33.67$a**	**$3.3\times10^3\pm24.00$a**

166
167 **Table 2: Effect of treatment on the fungi load of smoke-dried *C. gariepinus***

Dilution factor	Fresh	A	B	C
10^{-1}	31	14	24	16
10^{-2}	10	05	09	07
10^{-3}	03	02	05	02
Mean count	$1.4\times10^3\pm14.67$a	$8.8\times10^2\pm7.00$a	$2.1\times10^3\pm12.67$a	$9.5\times10^2\pm8.33$a

168
169
170 **Results of biweekly changes in the microbial load of smoke-dried *Clarias gariepinus***
171 **during ambient storage.**
172
173 After two (2) weeks of ambient storage**,** it was observed that there was no significant
174 difference ($p<0.5$) among the mean population of the bacteria and fungi. Sample A increased
175 from $1.3\times10^3\pm12.00$ to $1.6\times10^4\pm56.00$, sample B increased from $4.7\times10^3\pm33.67$ to
176 $2.6\times10^4\pm79.33$ and sample C increased from $3.3\times10^3\pm24.00$ to $1.7\times10^4\pm60.67$. For fungi
177 load, sample A increased from $8.8\times10^2\pm7.00$ to $2.2\times10^3\pm19.33$, sample B increased from
178 $2.1\times10^3\pm12.67$ to $4.7\times10^3\pm33.67$ and sample C increased from $9.5\times10^2\pm8.33$

to$3.1\times10^3\pm26.00$. Although sample B had the highest number of mean bacteria count compared to Sample A with the lowest mean count and sample B also had the highest number of fungi count when compared to Sample A (Tables 3 and 4).

After two (2) weeks of ambient storage, bacteria isolates from sample A were *Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Pseudomononas aruginosa* and *Micrococcus sp*, sample B were *Bacillus subtilis, , Streptococcus lactis, Enterobacter aerogenes, Proteus sp.,* and *Micrococcus sp* and sample C were *Bacillus subtilis, Streptococcus lactis, Enterobacter aerogenes, Pseudomononas aruginosa* and *Micrococcus sp*. The total bacteria frequency of occurrence and percentage after two weeks of storage of sample A increased to 26 and 83.87%, sample B increased to 43 and 79.62% and sample C increased to 28 and 80%. Fungi isolates from sample A were *Aspergillus sp, Penicillium sp, Fusarium sp, Mucor sp* and *Candida sp*, sample B also had *Aspergillus sp, Penicillium sp, Fusarium sp, Mucor sp* and *Candida sp* while sample C were *Aspergillus sp, Penicillium sp, Mucor sp* and *Candida sp.*

The total fungi frequency of occurrence and percentage after two weeks of storage for sample A was 5 and 16.15%, sample B was increased to 11 and 20.37% and sample C was reduced to 7 and 20% (Table 4).

After four (4) weeks of ambient storage, results show that means of bacteria population were significantly different as the results for sample B was significantly different for A and C. Sample A increased from $1.6\times10^4\pm56.00$ to $9.9\times10^4\pm100.7$, sample B increased from $2.6\times10^4\pm79.33$ to $1.1\times10^5\pm113.3$ and sample C increased from $1.7\times10^4\pm60.67$ to $9.6\times10^4\pm96.0$. For fungi mean count, Sample A increased from $2.2\times10^3\pm19.33$ to $7.9\times10^3\pm54.00$, sample B decreased from $4.7\times10^3\pm33.67$ to $1.1\times10^3\pm73.33$ and sample C increased from $3.1\times10^3\pm26.00$ to $8.0\times10^3\pm58.33$. Sample B had the highest bacteria and fungi mean count while sample C had the lowest bacteria count and sample A had the lowest fungi count as shown in Tables 3 and 4.

After four (4) weeks of ambient storage, bacteria isolates from sample A were *Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Pseudomononas aruginosa* and *Micrococcus sp*, sample B were *Bacillus subtilis, , Streptococcus lactis, Enterobacter aerogenes, Proteus sp.,* and *Micrococcus sp* and sample C were *Bacillus subtilis, Streptococcus lactis, Enterobacter aerogenes, Pseudomononas aruginosa* and *Micrococcus sp*.

The total bacteria frequency of occurrence and percentage after four (4) weeks of storage of sample A increased to 99 and 84.6%, sample B increased to 112 and 81.17% and sample C increased to 96 and 84.20%. Fungi isolates from sample A were *Aspergillus sp, Fusarium sp, Mucor sp* and *Candida sp*, sample B also had *Aspergillus sp, Penicillium sp, Fusarium sp, Mucor sp* and *Candida sp* while sample C were *Aspergillus sp, Penicillium sp, Mucor sp* and *Candida sp.*

The total fungi frequency of occurrence and percentage after two weeks of storage for sample A was reduced to 18 and 15.36% sample B was 26 and 18.84% and sample C was 18 and 15.825% (Table 3).

After six (6) weeks of ambient storage, the result of sample B was also significantly different from the mean population of sample A and C. For the bacteria population, sample A increased from $9.9\times10^4\pm100.7$ to $1.5\times10^5\pm153.3$, sample B increased from $1.1\times10^5\pm113.3$ to $1.7\times10^5\pm171.7$ and sample C increased from $9.6\times10^4\pm96.0$ to $1.5\times10^5\pm151.7$. For fungi population, sample A increased from $7.9\times10^3\pm54.00$ to $1.6\times10^4\pm98.7$, sample B increased from $1.1\times10^3\pm73.33$ to $2.3\times10^4\pm124.0$ and sample C decreased from $8.0\times10^3\pm58.33$ to $1.8\times10^3\pm105.3$. Sample B again had the highest bacteria and fungi mean count. Sample A and

C had similar bacteria mean count while sample A had the lowest fungi mean count of (Tables 5 and 6).

After six (6) weeks of ambient storage, bacteria isolates from sample A were *Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Pseudomononas aruginosa* and *Micrococcus sp*, sample B were *Bacillus subtilis, , Streptococcus lactis, Enterobacter aerogenes, Proteus sp.,* and *Micrococcus sp* and sample C were *Bacillus subtilis, Streptococcus lactis, Enterobacter aerogenes, Pseudomononas aruginosa* and *Micrococcus sp*. The total bacteria frequency of occurrence and percentage after six weeks of storage of sample A increased to 152 and 79.17%, sample B was increased to 150 and 76.13%, sample C increased to 96 and 84.20%. Fungi isolates from sample A were *Aspergillus sp, Fusarium sp,* and *Mucor sp*, sample B also had *Aspergillus sp, Penicillium sp, Fusarium sp, Mucor sp* and *Candida sp* while sample C were *Aspergillus sp, Penicillium sp, Mucor sp* and *Candida sp.* The total fungi frequency of occurrence and percentage after two weeks of storage for sample A was 40 and 20.83%, sample B reduced to 58 and 25.44% and sample C was 44 and 23.86% (Table 7).

Table 3: Biweekly changes in total estimated bacteria load of smoked-dried *C. gariepinus* during ambient storage

Dilution factor	Sample	Immediately after smoking	After two weeks of storage	After four weeks of storage	After six weeks of storage
	A	26	TNC	TNC	TNC
10^{-1}	B	67	TNC	TNC	TNC
	C	49	TNC	TNC	TNC
	A	07	69	TNC	TNC
10^{-2}	B	23	96	TNC	TNC
	C	15	75	TNC	TNC
	A	03	26	99	152
10^{-3}	B	11	43	112	170
	C	08	28	96	150
	A	$1.3\times10^{3}\pm12.00$a	$1.6\times10^{4}\pm56.00$a	$9.9\times10^{4}\pm100.7$a	$1.5\times10^{5}\pm153.3$a
Mean	B	$4.7\times10^{3}\pm33.67$a	$2.6\times10^{4}\pm79.33$a	$1.1\times10^{5}\pm113.3$b	$1.7\times10^{5}\pm171.7$b
	C	$3.3\times10^{3}\pm24.00$a	$1.7\times10^{4}\pm60.67$a	$9.6\times10^{4}\pm96.0$a	$1.5\times10^{5}\pm151.7$a

Table 4: Biweekly changes in total estimated fungi load of smoked-dried *C. gariepinus* during ambient storage

Dilution factor	Samples	Immediately after smoking	After two weeks of storage	After four weeks of storage	After six weeks of storage
	A	14	41	98	170
10-1	B	24	67	132	196
	C	16	52	108	178
	A	05	12	46	86
10-2	B	09	23	62	118
	C	07	19	49	94
	A	02	05	18	40
10-3	B	05	11	26	58
	C	02	07	18	44
	A	$8.8\times10^{2}\pm7.00$a	$2.2\times10^{3}\pm19.33$a	$7.9\times10^{3}\pm54.00$a	$1.6\times10^{4}\pm98.7$a
Mean	B	$2.1\times10^{3}\pm12.67$a	$4.7\times10^{3}\pm33.67$a	$1.1\times10^{3}\pm73.33$a	$2.3\times10^{4}\pm124.0$a
	C	$9.5\times10^{2}\pm8.33$a	$3.1\times10^{3}\pm26.00$a	$8.0\times10^{3}\pm58.33$a	$1.8\times10^{3}\pm105.3$a

DISCUSSION

Before storage of the fish samples, results showed that the fresh fish sample had the highest bacteria mean population count ($5.1\times103\pm46.00$) when compared with the treated samples, A ($1.3\times10^{3}\pm12.00$), B ($4.7\times10^{3}\pm33.67$) and C ($3.3\times10^{3}\pm24.00$). The total bacteria frequency of occurrence and percentage before storage for fresh fish sample was 11 and 78.56%, sample A was 7 and 58.33%, sample B was 23 and 71.89% and sample C was 15 and 68.18% respectively. These results may be due to unhygienic handling process during harvesting and transportation of the fish samples. The lowest mean count of fungi population was seen in sample A ($8.8\times10^{2}\pm7.00$) while the highest was sample B ($2.1\times10^{3}\pm12.67$) when compared with the fresh fish sample ($1.4\times10^{3}\pm14.67$) and sample C ($9.5\times10^{2}\pm8.33$). The total fungi frequency of occurrence and percentage before storage of the fresh fish sample were 3 and 23.45%, sample A was 5 and 41.67%, sample B was 9 and 28.11% and sample C was 7 and 31.82%. This shows that the process of smoking and the treatment with lemon grass and bay leaf marinade reduced the population of microorganisms in the fish samples. However the presence of microorganisms in the treated fish samples may be due to the handling procedure during smoking and the smoke-drying process in accordance with the findings of [14]who reported that lack of proper smoking and unhygienic handling of smoked fish products would result in a very high microbial load. This report is also similar to that of [11]

After two weeks of ambient storage, it was observed that there was no significant difference ($p<0.5$) among the mean population of the bacteria and fungi. Sample A increased from

$1.3\times10^3\pm12.00$ to $1.6\times10^4\pm56.00$, sample B increased from $4.7\times10^3\pm33.67$ to $2.6\times10^4\pm79.33$ and sample C increased from $3.3\times10^3\pm24.00$ to $1.7\times10^4\pm60.67$.

For fungi load, sample A increased from $8.8\times10^2\pm7.00$ to $2.2\times10^3\pm19.33$, sample B increased from $2.1\times10^3\pm12.67$ to $4.7\times10^3\pm33.67$ and sample C increased from $9.5\times10^2\pm8.33$ to $3.1\times10^3\pm26.00$. Although sample B had the highest number of mean bacteria counts compared to Sample A with the lowest mean count and sample B also had the highest number of fungi count when compared to Sample A. The total bacteria frequency of occurrence and percentage after two weeks of storage of sample A increased to 26 and 83.87%, sample B increased to 43 and 79.62% and sample C increased to 28 and 80%. The total fungi frequency of occurrence and percentage after two weeks of storage for sample A was 5 and 16.15%, sample B was increased to 11 and 20.37% and sample C was reduced to 7 and 20%. At this stage, there was an already observable swelling of the muscle of the fish samples thus the possibility of an increase in the moisture content of the smoked dried fish sample thereby enhancing the activity or proliferation of these micro-organisms. This is corroborated by [9] and [11] who said that smoked fish samples may have a relatively higher water activity level which is a prerequisite for microbial growth.

After four weeks of ambient storage, results show that means of bacteria population were significantly different as the results for sample B was significantly different for A and C. Sample A increased from $1.6\times10^4\pm56.00$ to $9.9\times10^4\pm100.7$, sample B increased from $2.6\times10^4\pm79.33$ to $1.1\times10^5\pm113.3$ and sample C increased from $1.7\times10^4\pm60.67$ to $9.6\times10^4\pm96.0$. For fungi mean count, Sample A increased from $2.2\times10^3\pm19.33$ to $7.9\times10^3\pm54.00$, sample B decreased from $4.7\times10^3\pm33.67$ to $1.1\times10^3\pm73.33$ and sample C increased from $3.1\times10^3\pm26.00$ to $8.0\times10^3\pm58.33$. Sample B had the highest bacteria and fungi mean count while sample C had the lowest bacteria count and sample A had the lowest fungi count. The total bacteria frequency of occurrence and percentage after four weeks of storage of sample A increased to 99 and 84.6%, sample B increased to 112 and 81.17% and sample C increased to 96 and 84.20%.The total fungi frequency of occurrence and percentage after two weeks of storage for sample A was reduced to 18 and 15.36% sample B was 26 and 18.84% and sample C was 18 and 15.825%. The increase in bacteria population may be as a result of high level of moisture content during storage in the fish samples. Bacteria thrives well where there is high moisture content but the decrease in the fungi mean population of sample B may be as a result of the reduction of moisture as fungi are saprophytes and proliferate in the absence of moisture as reported by [15] and [16]. Studies have shown that organic preservatives e.g moringa, lemon grass and bay leaf chloroform and ethanol extracts are potential sanitizers and or preservatives, this is because they were found to possess antimicrobial activities against some food borne microorganisms often implicated in spoilage of foods and food borne illness [17].

After 6 weeks of ambient storage, the result of sample B was also significantly different ($p>0.5$) from the mean population of sample A and C. For the bacteria population, sample A increased from $9.9\times10^4\pm100.7$ to $1.5\times10^5\pm153.3$, sample B increased from $1.1\times10^5\pm113.3$ to $1.7\times10^5\pm171.7$ and sample C increased from $9.6\times10^4\pm96.0$ to $1.5\times10^5\pm151.7$. For fungi population, sample A increased from $7.9\times10^3\pm54.00$ to $1.6\times10^4\pm98.7$, sample B increased from $1.1\times10^3\pm73.33$ to $2.3\times10^4\pm124.0$ and sample C decreased from $8.0\times10^3\pm58.33$ to $1.8\times10^3\pm105.3$. Sample B again had the highest bacteria and fungi mean count. Sample A and C had similar bacteria mean count while sample A had the lowest fungi mean count. The total bacteria frequency of occurrence and percentage after six weeks of storage of sample A increased to 152 and 79.17%, sample B was increased to 150 and 76.13%, sample C increased to 96 and 84.20%. The total fungi frequency of occurrence and percentage after two

weeks of storage for sample A was 40 and 20.83%, sample B reduced to 58 and 25.44% and sample C was 44 and 23.86%.

At this point, there were already observable signs of deterioration as a result of the muscle swelling due to increase in accumulation of moisture. This is in agreement with the findings of [18] who stressed that the microbial and chemical stability of fish and fish products during processing and storage is highly dependent in the water content of the product. Different parts of lemon grass and bay leaf plant contain different phenolics as well as rare combination of certain phytochemical compounds [17]. These compounds might be responsible for the significant decrease in the amount and variety of microorganisms isolated in the treated samples as opposed the control and brine-treated samples. Fish is a low-acid food [19] that can readily support the growth of pathogens, particularly bacteria if not properly handled and rapidly processed after harvesting. This partly explains why despite the fact that all the fish samples showed growth of heterotrophic bacteria and fungi throughout the study, the bacterial load was consistently higher than the fungal load.

[15] reported that bacteria are abundant in the diet and environment of fish and it is therefore impossible to avoid them. In the course of this study, 5 fungi genera were isolated from all the fish samples used. The bacteria identified comprised mainly of normal flora of fishponds and skin of fish processors [20]. The fungi isolates were identified as *Aspergillus sp, Penicillium sp, Fusarium sp, Mucor sp* and *Candida sp*. A total of 7 bacteria genera were also identified from the fish samples these include:*Bacillus subtilis, Escherichia coli, Streptococcus lactis, Enterobacter aerogenes, Proteus sp., Pseudomononas aruginosa* and *Micrococcus sp*. The microflora in the gut of the wild *Clarias gariepinus* was similar to those of [21] and [15]. [22] who gave similar reports of the occurrence of *E. coli* and *Pseudomonas sp* in smoked fish. *E. coli* and *Pseudomonas sp*are pathogens that can cause intestinal infections and nosocomial infections respectively in humans [23]. The occurrence of *Escherichia coli* in control samples is suggestive of faecal contamination of the water from which the fishes were reared because *E. coli* is an indicator organism and its presence in water or water products indicates the likely presence of faeces and by extension, the presence of other pathogenic intestinal microorganisms [24]. It is however noteworthy that contrary to the reports of [25] and [22], the common human pathogen *Listeria monocytogenes* was not detected in the fish samples. Another important pathogen, *Salmonella sp* was not found in any of the treated fish samples. This observation is in line with the report of [26] who showed activity of organic preservative hexane extract against *Salmonella, Shigella* and *Enterobacter*. However, this observation was contrary to the findings of [27] in which one of the bacteria isolated from smoked fish was *Salmonella sp*. The discrepancy in the present study and that of [27] may be attributed to proper aseptic techniques maintained at every stage of the experiments and the action of the treatment (lemon grass and bay leaf marinade). This result is corroborated by the report of [17] who reported similar microbial results due to the actions of organic preservatives. The fungi recovered in the fish samples have been reported to be regular contaminants of smoked fish [28].

Conclusion

In conclusion, results from this study showed that smoke-drying and spicing had significant effect on the microbial population and dynamics of the fish samples. The effect of smoke-drying and spicing with lemon grass and bay marinade reduced the growth level of microorganisms as the study revealed that all three smoked-dried fish sample treatment (A = lemon grass marinade, B = bay leaf marinade and C = lemon grass marinade + bay leaf marinade) had a relatively low bacterial and fungal count below the 5 x 10^5cfu/g recommended by the International Commission of Microbial Specification for Food and Food Products (ICMS, 2002). The Study also showed that sample B (bay leaf marinade) had more

microbial load than sample A (lemon grass marinade) and C (lemongrass marinade + bay leaf marinade).

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