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Studies on microbial load of *Clarias gariepinus* smoked with different parts and product of *Parkia biglobosa*

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ABSTRACT: This research aims to study the microbial load on *Clarias gariepinus* smoked using *Parkia biglobosa* parts and products. Forty (40) table-sized *Clarias gariepinus*, were smoked with ten pieces for each treatment, using *P. biglobosa* charcoal (PBC), bark (PBB), leaf (PBL), and wood (PBW). The smoked fish were stored for six (6) months to monitor the microbial load (bacteria and fungi). There was evidence of deterioration and increased microbial load as the storage time advanced. Fish with PBC had the highest Total Viable Count (TVC) of 1.01×10^5 cfu/g, whereas fish with PBW yielded the lowest value of 8.78×10^4 cfu/g. Also, the highest and lowest Total Coliform Counts (TCC) were observed in PBC-treated fish (4.10×10^4 cfu/g) and PBW-treated fish (2.13×10^4 cfu/g), respectively. Fish with PBL recorded the highest total fungi count of 9.59×10^5 cfu/g, while fish with PBW had the lowest count of 1.06×10^5 cfu/g. The culture-dependent approach of microorganism detection revealed the presence of *Klebsiela* spp and *Micrococcus* spp in PBC, *Bacillus* spp and *Staphylococcus aureus* in PBB, *Escherichia coli* and *Micrococcus* spp in PBL, and *Staphylococcus aureus* and *Bacillus* spp in PBW. The fungi species identified include *Aspergillus niger*, *Penicillium* sp., *Aspergillus flavus*, and *Aspergillus fumigatus.* The findings of this study suggest that PBW is better suited for smoking *C. gariepinus* due to its better resistance to bacterial and fungi load. Further research can be made to characterize the chemicals deposited by the smoke in different treatment of *Parkia biglobosa* (bark, leaf, wood, and charcoal).

Keywords: Bacteria, fungi, fish, preservation, smoking.

INTRODUCTION

In many countries, smoked dried fish is increasingly used to correct protein deficiency in the normal diet. It is relatively cheap and accessible (Ikutegbe and Sikoki, 2014). Fish is extremely perishable. It provides a great medium for microbial growth immediately after death (Ojutiku *et al*., 2009; Aliya *et al*., 2012; Oparaku and Mgbenka, 2012). Once spoilage sets in, the fish's odour, texture, colour and chemical composition change (Gupta and Gupta, 2006). Post-harvest fish losses in developing countries often exceed 50%, surpassing the losses of any other food commodity (Olatunde, 1998). Researchers have employed different methods to reduce microbial spoilage and extend the shelf life of fish, including smoking, drying, freezing, salting, canning, and so on (Awan and Okaka, 1985; Gupta and Gupta, 2006). Among the different preservation methods employed for a long period, smoking is the simplest and easy-going method as it does not require refined or sophisticated equipment (Olayemi *et al.*, 2011). Smoking improves the shelf-life of smoked fish quite better than other forms of preservation because of the reduced moisture content (<30%) (Eyo, 2001); also, smoking inhibits microbial growth in stored fish products (Salan *et al.,* 2006).

Microbial action plays a large part in the spoilage of fish and fish products (Eyo, 2001; Agbolagba and Iyeru, 1998). The exposure of fish to dust, microbial, and other environ-

mental contaminants results in spoilage. Enzymatic tissue breakdown initiates the deterioration of fish products, and microorganisms proliferate and penetrate the fish flesh as the process continues (Garba *et al*., 2021). Many scientists have reported microbial load associated with smoked fish. *S. aureus, Pseudomonas, Streptococcus, Bacillus,* (Bacteria) and Yeast*, Aspergillus niger* and *Penicillium* spp. (fungi) were reported to be isolated (Daniel *et al.,* 2013) on microbial diversity of smoked fish sold in Benin City, Nigeria. Bukola *et al.* (2008) recorded fungal isolates found in smoked dried fishes sold in different markets in Uyo, Nigeria as follows *Aspergillus flavus, Aspergillus tereus, Aspergillus fumigatus, Absidia* spp., *Rhizopus* spp., *A. niger, Mucor* spp., *Cladosporum* spp., *Penicillium italicum, Penicilium viridatus, Candida tropical* and *Fusarium moniliformis.* Akise *et al.* (2013) reported major fungi species isolated from smoke-dried fish under storage including *P. italicum*, *Penicillium oxalicum*, *Mucor, Saccharomyces, Rhodotorula* spp. and *Aspergillus* spp. Majority of the fungi produce mycotoxins. *Aspegillus* species are known to produce mycotoxins such as aflatoxins, ochratoxins and sterigmatocystin (Hashem, 2011). Fafioye *et al.* (2002) isolated and identified eleven different fungal species, of which *Aspergillus flavus* was the most frequently encountered fungus on the fish species on the fungal infestation of five traditionally smoked dried freshwater fish in Ago-Iwoye, Nigeria. According to Aberoumand (2010), *Escherichia coli* is an example of enteric bacteria which causes gastroenteritis. *E. coli and Staphylococcus aureus* were reported as the predominant microorganisms in smoked fish in Delta state, Nigeria (Okonta and Ekelemu, 2005).

Also, researchers have identified some natural plants, such as Neem Seed which showed promising characteristics for the control (Ajao, 2012). Due to the perishable nature of aquaculture products, the choice of preservation method must significantly halt microbial spoilage tendencies. *Parkia biglobosa* is also known as African locust beans, and it is found in a wide range of environments in Africa, also is a dominant tree in the middle belt of Nigeria. It is highly medicinal and economic tree. Abioye *et al.* (2013) reported its potential in managing bacterial infections. This research is aim at to study how the use of various parts and products of *Parkia biglobosa* (leaf, bark, wood, and charcoal) for smoking *C. gariepinus* affects the microbial load of the product. The fish species (*C. gariepinus*) used in this study is acceptable, commonly consumed, and widely cultured in Nigeria.

MATERIALS AND METHODS

Experimental site

This study was conducted at the Department of Fisheries and Aquaculture, Joseph Sarwuan Tarka University Makurdi, Benue State, Nigeria. Its geographical coordinates lie at latitude 7° 44' North and longitude 8°35' East. It is located in the southern guinea savannah area of Nigeria and consists of two different seasons (dry and wet). The higest rainfall in the area annually experienced between the month of April and October with a mean value of 1137 mm (Isikwe and Onyilo, 2010)

Sample collection area

Table-sized *C. gariepinus* with mean weight of 814.26 g obtained from Wadata Market in Makurdi (7°44' 42.36" N and 8°30' 45.36" E) were transported to the Department of Fisheries and Aquaculture, Joseph Sarwuan Tarka University Makurdi, in an ice box. Forty pieces (40) were obtained, ten (10) pieces for each treatment (bark, leave, wood and charcoal of *P. biglobosa*).

Smoking process

The fish sample were gutted and washed thoroughly with clean water. The cleaned fish samples were then kept on racks (protected by a mosquito net to prevent perching by flies) to first drain before placing them in six drum-type smoking kilns (two for each treatment) (Yola and Timothy, 2012). Each of the smoking kilns has *P. biglobosa* leaf (PBL), *P. biglobosa* bark (PBB), *P. biglobosa* charcoal (PBC), and *P. biglobosa* wood (PBW) as the fuel sources and each treatment replicated. The maximum temperature in the smoking chamber was monitored and controlled to prevent burning; the fish were periodically turned to achieve uniform smoking. The fish sample were smoked for two (2) days first to be smoked dried was charcoal, wood, bark and leave respectively. After smoke drying the fish, they were packed in a woven mesh wire basket (conventional storage practiced in sub-Saharan Africa) to monitor the sample's microbial load every month for six (6) months.

Microbial analysis

Microbial analysis was conducted at the Food Microbiology Laboratory, Department of Food Science Technology, Joseph Sarwuan Tarka University Makurdi, Benue State, Nigeria.

Sample preparation for microbial analysis

The samples were aseptically packaged for microbial load in sterile plastic bags. The tissues from each treatment were cut and prepared by crushing fish muscle in a sterile mortar. From the sample, 1 g of each was weighed out and

homogenized (homogenization was to obtain uniform distribution of cells) in a sterile test tube containing 9 ml of distilled water which became the stock solution (1:10 dilution). Serial dilutions of up to 10^{-9} and used 10 ml for plate inoculations.

Media used

The media used are Mackonkey Agar (MCA), Nutrient Agar, and Sabourand Dextrose Agar (SDA). All media were prepared according to the manufacturer's specifications.

Inoculation of media plates

The pour-plate method was used to inoculate all media plates. A loopful of each stock solution were picked using sterilized wire loop and streaked across the surface of each media plate. The inoculums were spread by gently swirling the plate. Afterwards, the plates were incubated at 37ºC for 24 hours and then colonies were observed and recorded. Each picked discrete colony was sub-cultured on fresh media plates to obtain pure cultures. Proper labelling was applied to all plates and test tubes used. Bacterial growths from the plates underwent Gram staining, motility, and biochemical tests. Each sample's total viable bacteria count was estimated using the method described by Collin and Lyne (1970). The mould count method of Harrigan and Maccance (1976) was adopted to enumerate yeast and mold.

Biochemical test

The following biochemical tests were carried out: motility, indole, gram reaction, catalase, coagulate, citrate, oxidase, H_2S, following the method of Cheesbrough (2002).

Identification of microorganisms

Features such as spore and hyphae morphology were observed and compared with standard atlas (Ochei and Kohatkar, 2000).

Formulae used for calculation of counts

The method described by Collins and Lyne (1970) was used to estimate the total viable count of the samples. Countable plates showing 20-100 colonies were selected and counted. The mean of the duplicate count on each given dilution was used to estimate the total viable count for the sample in colony-forming units per gram (cfu/g).

Calculation of colony forming unit per gram:

Let the dilution factor be (10^{-a} –10^{-9})

Let the average number of colonies per dilution be = C.

$$Total\ viable\ count\ per\ gram = dilution\ factor\ \times colonies\ counted$$

$$= 1/D\ \times C = C/D\ (cfu.g^{-1})$$

Calculation of average sum of means (cfu/g^{-1}):

Let the arithmetic mean of the set of cfu.g^{-1} value N be denoted by X and it is defined as:

$$X = \sum x/_N$$

Where $\sum x$ = Sum of the mean of the total (cfu.g^{-1}) of the entire individual sample, N = Number of samples tested. Total bacteria count was reported (visible counts and total counts) and calculated as

$$Total\ microbial\ count\ (V) = N/VXD$$

Where V = viable count (cfu/g^{-1}), N = Number of colonies, V = Volume transferred into petri dishes, D = Dilution factor.

Statistical analysis

Data were subjected to statistical analysis using analysis of variance (ANOVA) at a 5% significance level, and significantly different means were separated using Fisher's LSD.

RESULTS

Microbial analysis of smoked *Clarias gariepinus* using *Parkia biglobosa* (charcoal, bark, leaf, and wood)

Tables 1 to 4 display the results of a microbiological examination of smoked *C. gariepinus* prepared with charcoal, bark, leaf, and wood from *Parkia biglobosa*. The result shows a significant difference ($p<0.05$) in the total bacteria count, coliform count, and fungi count.

Table 1 shows the microbial analysis of stored *Clarias gariepinus* smoked using charcoal of *Parkia biglobosa*. From the result obtained, there was an increase in the total viable count (0.00 to 2.20×10^5 cfu/g), total coliform count (0.00 to 1.50×10^5 cfu/g) and total fungal count (0.00 to

Table 1. Microbial load of stored *Clarias gariepinus* smoked using charcoal of *Parkia Biglobosa.*

Time (month)	TVC (cfu/g)	CC (cfu/g)	FC (cfu/g)
Initial (May)	0.00	0.00	0.00
June	$3.45\times10^{3}\pm0.01^{a}$	0.00	$4.1\times10^{3}\pm0.00^{a}$
July	$2.05\times10^{4}\pm0.01^{b}$	0.00	$3.10\times10^{4}\pm0.01^{b}$
Aug	$1.25\times10^{5}\pm0.05^{c}$	$3.20\times10^{3}\pm0.00^{a}$	$3.65\times10^{4}\pm0.01^{b}$
Sept.	$1.55\times10^{5}\pm0.05^{d}$	$1.85\times10^{4}\pm0.01^{b}$	$3.95\times10^{4}\pm0.01^{b}$
Oct.	$1.85\times10^{5}\pm0.05^{e}$	$1.15\times10^{5}\pm0.05^{c}$	$3.35\times10^{5}\pm0.05^{c}$
Nov.	$2.20\times10^{5}\pm0.00^{f}$	$1.50\times10^{5}\pm0.00^{d}$	$3.65\times10^{5}\pm0.05^{d}$
P-Value	0.000	0.000	0.000

Means in the same column with different superscripts differ significantly (P<0.05). **Key:** TVC (Total Viable Count), CC (Coliform Count) FC (Fungal Count).

Table 2. Microbial load of stored *Clarias gariepinus* smoked using bark of *Parkia biglobosa.*

Time (month)	TVC (cfu/g)	CC (cfu/g)	FC (cfu/g)
Initial(May)	0.00	0.00	0.00
June	$2.85\times10^{3}\pm0.00^{a}$	0.00	$2.65\times10^{4}\pm0.02^{a}$
July	$3.15\times10^{4}\pm0.01^{b}$	0.00	$3.40\times10^{4}\pm0.01^{ab}$
Aug	$1.15\times10^{5}\pm0.05^{c}$	$3.40\times10^{3}\pm0.00^{a}$	$4.25\times10^{4}\pm0.01^{b}$
Sept.	$1.45\times10^{5}\pm0.05^{d}$	$2.15\times10^{4}\pm0.01^{b}$	$4.60\times10^{4}\pm0.01^{b}$
Oct.	$1.85\times10^{5}\pm0.05^{e}$	$1.15\times10^{5}\pm0.05^{c}$	$3.80\times10^{5}\pm0.00^{c}$
Nov.	$2.05\times10^{5}\pm0.05^{f}$	$1.40\times10^{5}\pm0.00^{d}$	$4.10\times10^{5}\pm0.10^{d}$
P-Value	0.000	0.000	0.000

Means in the same column with different superscripts differ significantly (p<0.05). **Key:** TVC (Total Viable Count), CC (Coliform Count) FC (Fungal Count).

Table 3. Microbial load of stored *Clarias gariepinus* smoked using leaf of *Parkia biglobosa.*

Time (month)	TVC (cfu/g)	CC (cfu/g)	FC (cfu/g)
Initial (May)	0.00	0.00	0.00
June	$3.05\times10^{3}\pm0.00^{a}$	0.00	$3.60\times10^{3}\pm0.00^{a}$
July	$1.70\times10^{4}\pm0.01^{b}$	0.00	$2.45\times10^{4}\pm0.01^{b}$
Aug	$1.15\times10^{5}\pm0.05^{c}$	$2.65\times10^{3}\pm0.00^{a}$	$2.85\times10^{4}\pm0.01^{bc}$
Sept.	$1.45\times10^{5}\pm0.05^{d}$	$3.40\times10^{3}\pm0.00^{a}$	$3.45\times10^{4}\pm0.02^{c}$
Oct.	$1.70\times10^{5}\pm0.00^{e}$	$1.85\times10^{4}\pm0.01^{b}$	$2.70\times10^{5}\pm0.05^{d}$
Nov.	$1.95\times10^{5}\pm0.05^{f}$	$1.28\times10^{5}\pm0.03^{c}$	$3.05\times10^{5}\pm0.05^{e}$
P-Value	0.000	0.000	0.000

Means in the same column with different superscripts differ significantly (P<0.05). **Key:** TVC (Total Viable Count), CC (Coliform Count) FC (Fungal Count).

3.65×10^{5} cfu/g) with the increase in storage period (June to November).

Table 2 shows the microbial analysis of *Clarias gariepinus* smoked using the bark of *Parkia biglobosa,* from the result. An increase was observed in the total bacterial count, ranging from 0.00 to 2.05×10^{5} cfu/g, total coliform count (0.00 to 1.40×10^{5} cfu/g), and total fungal count (0.00 to 4.10×10^{5} cfu/g) of the smoked *Clarias gariepinus* with an increase in the storage period (June to November).

The result in Table 3 shows that the microbial analysis of *Clarias gariepinus* smoked using the leaf of *Parkia biglobosa*, which showed that there was an increase in the total bacterial count (0.00 to 1.95×10^{5} cfu/g), total coliform count (0.00 to 1.28×10^{5} cfu/g) and total fungal count (0.00 to 3.05×10^{5} cfu/g) of the smoked *Clarias gariepinus* with

Table 4. Microbial load of stored *Clarias gariepinus* smoked using wood of *Parkia biglobosa.*

Time (month)	TVC (cfu/g)	CC (cfu/g)	FC (cfu/g)
Initial(May)	0.00	0.00	0.00
June	$3.05\times10^{3}\pm0.00^{a}$	0.00	$3.75\times10^{3}\pm0.00^{a}$
July	$1.75\times10^{4}\pm0.01^{b}$	0.00	$2.65\times10^{4}\pm0.01^{b}$
Aug	$1.15\times10^{5}\pm0.05$c	$3.10\times10^{3}\pm0.00^{a}$	$3.25\times10^{4}\pm0.01^{bc}$
Sept.	$1.40\times10^{5}\pm0.00^{d}$	$3.75\times10^{3}\pm0.00^{a}$	$3.55\times10^{4}\pm0.01^{c}$
Oct.	$1.55\times10^{5}\pm0.05^{e}$	$1.75\times10^{4}\pm0.01^{b}$	$3.15\times10^{5}\pm0.05^{d}$
Nov.	$1.83\times10^{5}\pm0.03^{f}$	$1.25\times10^{5}\pm0.05^{c}$	$3.30\times10^{5}\pm0.00^{e}$
P-Value	0.000	0.000	0.000

Means in the same column with different superscripts differ significantly ($p<0.05$). **Key:** TVC (Total Viable Count), CC (Coliform Count) FC (Fungal Count).

Table 5. Mean microbial load of stored *Clarias gariepinus* Smoked using charcoal, bark, leaf and wood of *Parkia biglobosa.*

Treatment	TVC (cfu/g)	CC (cfu/g)	FC (cfu/g)
Charcoal	$1.01\times10^{5}\pm2.37$	$4.10\times10^{4}\pm1.64$	$1.16\times10^{5}\pm4.13$
Bark	$9.78\times10^{4}\pm2.22$	$4.00\times10^{4}\pm1.56$	$1.34\times10^{5}\pm4.60$
Leaf	$9.22\times10^{4}\pm2.15$	$2.17\times10^{4}\pm1.21$	$9.59\times10^{5}\pm3.43$
Wood	$8.76\times10^{4}\pm2.01$	$2.13\times10^{4}\pm1.89$	$1.06\times10^{5}\pm3.81$
P-Value	0.972	0.618	0.920

Means in the same column with different superscripts differ significantly ($p<0.05$). **Key:** TVC (Total Viable Count), CC (Coliform Count) FG (Fungal Count).

an increased in the storage period (June to November).

Table 4 shows the microbial analysis of *Clarias gariepinus* smoked using wood of *Parkia biglobosa*, the result showed that there was an increase in the total bacterial count (0.00 to 1.85×10^{5} cfu/g), total coliform count (0.00 to 1.25×10^{5} cfu/g) and total fungal count (0.00 to 3.30×10^{5} cfu/g) of the smoked *Clarias gariepinus* with an increased in the storage period (June to November).

Table 5 presents the mean microbial load of *C. gariepinus* smoked using different parts of *P. biglobosa* (charcoal, bark, leaf, and wood). The result revealed the highest and the lowest value of the microbial load (TVC) in charcoal (1.01×10^{5} cfu/g) and wood (8.78×10^{4} cfu/g). The highest and lowest total coliform count was observed in charcoal (4.10×10^{4} cfu/g) and wood (2.13×10^{4} cfu/g), respectively. The highest total fungi count was obtained in leaf (9.59×10^{5} cfu/g), while the lowest was obtained in wood (1.06×10^{5} cfu/g).

Table 6 present the result of bacterial isolate from all the treatment of smoke dried *Clarias gariepinus* with *Parkia biglobosa. Klebsiela* spp and Micrococcus spp Charcoal, *Bacillus* spp and *Staphylococcus aureus* in Bark, *Escherichia coli* and *Micrococcus* spp in Leave, and *Staphylococcus aureus* and *Bacillus* spp in Wood.

Table 7 shows the result of fungi isolate from all the treatment of smoke dried *Clarias gariepinus* with *Parkia biglobosa.* Species identified include Charcoal (*Aspergillus niger* and *Aspergillus flavus),* Bark (*Aspergillus flavus, Penicillium* sp. And *Aspergillus niger),* Leave (*Aspergillus flavus, Penicillium* sp. And *Aspergillus niger)* and Wood (*Penicillium* sp. *Aspergillus flavus*, and *Aspergillus fumigatus).*

DISCUSSION

The study revealed *Clarias gariepinus* smoked with *P. biglobosa* parts and product (bark, leaf, wood, and charcoal), wood treatment had the lowest bacterial and fungal load. Charcoal exhibited the highest bacterial load, while the leaves showed the highest fungal load in smoked fish. The presence of five (5) bacteria species and four (4) fungal species were isolated and identified from fish sample treated with different parts and product of *P. biglobosa.Klebsiela* sp. and *Micrococcus* sp. was found in PBC*, Bacillus* sp. and *Staphylococcus aureus* in PBB*, Escherichia coli* and *Micrococcus* sp. in PBL, and *Staphylococcus aureus* and *Bacillus* sp. in PBW. The fungi species identified included *Aspergillus niger*, *Penicillium*, *Aspergillus flavus*, and *Aspergillus fumigatus.*

The bacterial and fungal load of the smoked stored fish (*Clarias gariepinus*) increases as the length of the storage

Table 6. Microbiological and biochemical characteristics of bacterial isolates from smoked *Clarias gariepinus* using *Parkia biglobosa* (charcoal, bark, leaf and wood).

Sample	Cell morphology	Gram Rxn.	Motility	Catalase	Coagulase	Citrate	Oxidase	Indole	H_2S	Possible microorganism
Charcoal	Rods	-	-	-	-	+	-	-	-	*Klebsiela* sp.
	Cocci	+	-	+	-	-	-	-	-	*Micrococcus* sp.
Bark	Rods	+	+	+	-	-	-	-	-	*Bacillus* sp.
	Cocci	+	-	+	+	-	-	-	-	*S. aureus*
Leaf	Rods	-	+	+	-	-	-	+	-	E. *coli*
	Cocci	+	-	+	-	-	-	-	-	*Micrococcus* sp
Wood	Rods	+	+	+	-	-	-	-	-	*S. Aureus*
	Cocci	+	-	+	+	-	-	-	-	*Bacillus* sp.

Table 7. Morphological characteristics of fungal isolates from smoked *Clarias gariepinus* using *Parkia biglobosa* (charcoal, bark, leaf and wood).

Samples	Colony	Microscopic description	*Organism*
Charcoal	Jet-back conidia Green-gray, white Aperon at the margin	Branched conidia in chain Conidiophose with vesicle	*Aspergillus niger* *Aspergillus famigatus*
Bark	Jet-back conidia Yellow-green mycelium Green-gray, white Aperon at the margin	Branched conidia in chain Septate branching Conidiophose with vescle	*Aspergillus flavus* *Penicilium spp* *A. niger*
Leave	Greenish mycelium Yellow-green mycelium Jet-black conidia	Unbranched conidiophose in chain Septate branching Branched conidia in chain	*A. flavus* *Penicilium spp* *A. niger*
Wood	Yellow-green mycelium Jet-black conidia Greenish mycelium	Septate branching Branched conidia in chain Unbranched conidiophose in chain	*Penicillium spp* *A. niger* *A. flavus*

period. This is similar to the findings obtained by Dutta *et al*. (2018). The final value of microbial load in this study falls within the maximum recommended range (5 x 10^5 cfu/g) of bacteria count for good quality fish products as recommended by Microbiological Guidelines for ready-to-eat food (i.e. < 10^6) by Centre for Food Safety, Food and Enviromental Hygiene Department (2007). The spoilage in the smoked fish sample is attributed to increased microbial load and storage length since microbes play a vital role in the spoilage of fish and fish products (Eyo, 2001).

Ayeloja *et al.* (2013) also reported similar microbes to those identified in the present study. Some bacteria are commonly associated with fish under storage conditions (Abolagba and Igbinevbo, 2010; Emikpe *et al.,* 2011). *Bacillus* sp. is a normal microbial flora of fish, which is not harmful but could become pathogenic in some environments.

The presence of *Staphylococcus aureus and Escherichia coli* recorded in this study is in agreement with the work by Ayuba *and* Omeji (2006) and Garba *et al.* (2021) and who reported the presence of the same microbes in smoked dried sardine and smoked *C. gariepinus. E. coli* could be associated with improper handling of the fish product during smoking, which agrees

with Ayulo *et al.* (1994). Asai *et al.* (1999) stated that *E. coli* is associated with improper fish handling. It is also an enteric bacteria causing gastroenteritis and the contamination of fish or fish products with pathogenic and faecal contamination (Feng, 2002). *Staphylococcus aureus* produces enterotoxins that cause gastroenteritis (Novotny *et al.* 2004), thus possibly causing food poisoning and food-borne diseases that could result from consuming contaminated smoked fish. Researchers have reported the presence of *Escherichia coli, Proteus spp, Micrococcus spp,* and *Bacillus* sp in smoked freshwater prawns (Akinyemi 2013).

Several fungi identified in the stored smoked *C. gariepinus* in this study (i.e., *A. niger*, *Penicillium* spp. *A. fumigatus* and *A. flavus*) were also found growing on smoked freshwater prawn (*Macrobrachium* sp.) as reported by Akinyemi (2013). *Aspergillus* sp. causes food spoilage and poisoning, and its introduction onto the stored fish could have been a result of post-processing contamination according to the assertion of Hohl and Feldmesser (2007).

The microbial loads of all the treatments differ. This difference could be due to the active chemical ingredients in the smoke from the different energy sources. This report aligns with Guillén and Manzanos (2002), who stated that the differences in mould attack could be due to differences in active microbial components in the respective smoke sources. The study by Bhattacharyya *et al.* (2007) and Oduor-Odote *et al.* (2010) on Neem plant wood revealed that the smoke probably contains active antifungal ingredients, which are deposited on the fish muscle during processing and subsequently reduce attack by mould during storage. The variations in mould infestation of stored smoked fish may be linked to the species of wood tree used for smoking (Oduor-Odote *et al*., 2010).

Conclusion/Recomendation

This study showed that microbial load of all the smoked stored *Clarias gariepinus* increased with an increase in the storage period but were quantitatively within safe consumable ranges. *Parkia biglobosa* wood had superior characteristics regarding resistance to microbial (bacteria and fungi) attacks. Further research can be made to characterize the chemicals deposited by the smoke in different treatment of *Parkia biglobosa* (bark, leaf, wood, and charcoal).

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Aberoumand, A. (2010). Estimation of microbiological variations in minced lean fish products. *World Journal of Fish and Marine Sciences*, *2*(3), 204-207.

Abioye, E. O., Akinpelu, D. A., Aiyegoro, O. A., Adegboye, M. F., Oni, M. O., & Okoh, A. I. (2013). Preliminary phytochemical screening and antibacterial properties of crude stem bark extracts and fractions of Parkia biglobosa (Jacq.). *Molecules*, *18*(7), 8485-8499.

Abolagba, O. J., & Igbinevbo, E. E. (2010). Microbial load of fresh and smoked fish marketed in Benin metropolis, Nigeria. *Research Journal of Fisheries and Hydrobiology*, *5*(2), 99-104.

Agbolagba, O. J., & Iyeru, O. A. (1998). Study of insect pest infecting traditionally processed fish sold in Benin City metropolis. *Nigerian Journal of Applied Science, 16, 25*, *29*.

Ajao, M. (2012). Evaluation of the efficacy of Neem Seed Oil (NSO) for the control of *Dermestes maculates* Degeer, 1774 coleoptera: Dermestidae in *Clarias gariepinus* (Burchell 1822) (*pisces: claridae*) *Munis. Entomology and Zoology Journal*, *7*(2), 188-194.

Akinyemi, A. A. (2013). Micro–organisms associated with smoked cured fresh water prawn in open markets in Abeokuta, Nigeria. *Journal of Environmental Sciences and Resources Management*, *5*(2), 42-53.

Akise, O. G., Abolagba, O. J., & Eyong M. M. (2013) Mycoflora of three fish species smoke-dried using rubber wood (*Hevae brassillensis*) in Nigeria. *Greener Journal of Agricultural Science,* 3(5), 396-402.

Aliya, G., Humaid, K., Nasser, A., Sami, G., Aziz, K., Nashwa, M., & Ponnerassery, S. S. (2012). Effect of the freshness of starting material on the final product quality of dried salted shark. *Advance Journal of Food Science and Technology,* 4(2), 60-63.

Asai, Y., Murase, T., Osawa, R., Okitsu, T., Suzuki, R., Sata, S., Yamai, S., Terajima, J., Izumiya, H., Tamura, K., & Watanabe, H. (1999). Isolation of Shiga toxin-producing *Escherichia coli* O157: H7 from processed salmon roe associated with the outbreaks in Japan, 1998, and a molecular typing of the isolates by pulsed-field gel electrophoresis. *Kansenshogaku zasshi. The Journal of the Japanese Association for Infectious Diseases*, *73*(1), 20-24.

Awan, I. A., & Okaka, I. C. (1985). *Element of food spoilage and preservation*. Snaap Press Enugu, Nigeria. Pp. 60-70.

Ayeloja, A. A., George, F. O. A., Akinyemi, A. A., Jimoh, W. A., Dauda, T. O., & Akinosho, G. A. (2013). Effect of processing methods on nutritive value of catfish (*Clarias gariepinus*). *Food Science and Quality Management*, *11*, 31-38.

Ayuba, V. O., & Omeji, N. O. (2006). Effect of insect infestation on the shelf life of smoked dried fish. *Proceedings of the 21st Annual Conference of the Fisheries Society of Nigeria (FISON), Calabar, 13th-17th November,* 2006. Pp. 357-359.

Ayulo, A. M, Machado R. A., & Scussel, V. M. (1994). Entero-toxigenic *Escherichia coli* and *Staphylococcus aures* in fish and sea food from the Southern region of Brazil. *International Journal of Food and Microbiology,* 24,171-178.

Bhattacharyya, N., Chutia, M., & Sarma, S. (2007). Neem (Azadirachta indica A. Juss), a potent biopesticide and medicinal plant: A review. *Journal of Plant Sciences*, *2*(3), 251-259.

Bukola, C. A., Abiodun, A. O., & Ukpe, G. P. (2008). Mycofloral of smoke-dried fishes sold in Uyo, Eastern Nigeria. *World Journal of Agricultural Sciences*, *4*(3), 346-350.

Centre for Food Safety, Food and Enviromental Hygiene

Department (2007). Microbiological Guideline for Ready to–eat–Food. Centre for Food Safety, Food and Enviromental Hygiene Department. 43/F, Queensway Government Offices, 66 Queensway, Hong Kong.

Cheesbrough, M. (2005). *District laboratory practice in tropical countries, part 2*. Cambridge university press. Pp. 62-70.

Collin, C. H., & Lyne P. M. (1970). *Microbial methods*. Published by Butterworths.

Daniel, E. O., Ugweze A. U., & Igbegu H. E. (2013). Microbiological quality and some heavy metal analysis of smoked fish sold in Benin City, Edo State Nigeria. *World Journal Fish Marine Science*, 5(3), 239-243.

Dutta, M., Majumdar, P. R., Rakeb-Ul-Islam, M. D., & Saha, D. (2018). Bacterial and fungal population assessment in smoked fish during storage period. *Journal Of Food Microbiology Safety and Hygiene*, *3*(127), 2476-2059.

Emikpe, B. O., Adebisi, T., & Adedeji, O. B. (2011). Bacteria load on the skin and stomach of *Clarias gariepinus* and *Oreochromis niloticus* from Ibadan, South West Nigeria: Public health implications. *Journal of Microbiology and Biotechnology Research*, *1*(1), 52-59.

Eyo, A. A. (2001). *Fish processing technology in the tropics*. National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Nigeria. 403p.

Fafioye, O. O., Efuntoye, M. O., & Osho, A. (2002). Studies on the fungal infestation of five traditionally smoke-dried freshwater fish in Ago-Iwoye, Nigeria. *Mycopathologia*, *154*, 177-179.

Feng, P., Weagant S. D., Grant M. A., & Burhardt W. (2002). Enumeration of *E. coli* and coliform bacteria in bacteriological analytical manual (8th edition) R. I. Merker FDA (United State Food and Drugs Administration)/Centre for Food Safety and Applicant Nutrition.

Garba, A. A., Ochogwu, J., Usman, B. I., & Adadu, M. O. (2021). Assessment of the extent of microbial load of smoked dried African catfish (*Clarias gariepinus*) sold in Maiduguri markets. *International Journal of Fisheries and Aquatic Studies*, 9(3), 278-287.

Guillén, M. D., & Manzanos, M. J. (2002). Study of the volatile composition of an aqueous oak smoke preparation. *Food Chemistry*, *79*(3), 283-292.

Gupta, S. K., & Gupta, P. C. (2006). *General and Applied Ichthyology (Fish and Fisheries)*. S.Chand and Co. Ltd, Ram Nagar, New Dehli. Pp. 1045-1068.

Hashem, M. (2011). Isolation of mycotoxin-producing fungi from fishes growing in aquacultures. *Research Journal of Microbiology*, *6*(12), 862.

Hohl, T. M., & Feldmesser, M. (2007). *Aspergillus fumigates*: Principles of pathogenesis and host defence. *Eukaryotic Cell,* 6(11), 1953-1963.

Ikutegbe, V., & Sikoki, F. (2014). Microbiological and biochemical spoilage of smoke-dried fishes sold in West African open markets. *Food Chemistry*, *161*, 332-336.

Isikwe, M. O., & Oyilo, A. F. (2010). Influence of land use on the hydraulic response of loamy sand tropical soil. *Journal of Engineering and Applied Science*, *6*(2), 145-150.

Novotny, I., Dvorska, L., Lorencova, A., Beran, V., & Pavlik, I. (2004). Fish a potential source of bacterial pathogens for human beings. Veterinary Medicine Czech, *49*(9), 343-358.

Ochei, J., & Kolhatkar, A. A. (2000). Medical mycology: In *Medical Laboratory Science, Theory and Practice*. Tata-McGraw Hill, 7 West Patel Nagar New Delhi. Pp. 1047-1050.

Oduor-Odote, P. M., Obiero, M., & Odoli, C., Oduor-Odote, P. (2010), organoleptic effect of using different plant materials on smoking of marine and freshwater catfish. *African Journal of Food Agriculture Nutrition and Development.* 10(6), 2658-2677.

Ojutiku, R. O., Kolo, R. J., & Mohammed, M. L. (2009). Comparative study of sun drying and solar tent drying of Hyperopisus bebeoccidentalis. *Pakistan journal of Nutrition*, *8*(7), 955-957.

Okonta, A., & Ekelemu, J. (2005). A preliminary study of microorganisms associated with fish spoilage in asaba, southern Nigeria, proceedings of 20th annual conference of the Fisheries Society of Nigeria (FISON). Pp. 557-560.

Olatunde, A. A. (1998). Approach to the study of fisheries biology in Nigeria inland water proceedings of international conference of two decade of reserch in lake kaiji. Pp. 338-542.

Olayemi, F. F., Adedayo, M. R., Bamishaiye, E., & Awagu, E. F. (2011). Proximate composition of catfish (*Clarias gariepinus*) smoked in Nigerian Stored Products Research Institute (NSPRI) developed kiln. *International Journal of Fisheries and Aquaculture*. 3(5), 96-98.

Oparaku, N. F.. & Mgbenka, B. O. (2012). Effects of electric oven and solar dryer on a proximate and water activity of *Clarias gariepinus* Fish. *European Journal of Science Research, 81*(1), 139-144.

Salan, O. E, Juliana, A. G., & Marilia, O. (2006), Used of smoking to add value to *Salmoned trout*. *Brazilian* Archives of *Biology* and *Technology, 49*(1), 57-62.

Yola, I. A., & Timothy, O. (2012). Proximate composition and consumer acceptability of African mudfish clarias gariepinus smoked with two energy sources. *Bayero Journal of Pure and Applied Sciences, 5*(2), 115-118.