E-ISSN: 2663-1067
P-ISSN: 2663-1075
https://www.hortijournal.com
IJHFS 2023; 5(2): 35-44
Received: 21-08-2023
Accepted: 25-09-2023

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Effects of chemical preservatives and liquid smoke on the microbial quality of smoked and oven dried tilapia fish (*Oreochromis niloticus*)

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DOI: https://doi.org/10.33545/26631067.2023.v5.i2a.176

Abstract
Fish is a major source of protein and its degradation keeps causing post- harvest losses and a decline in fish supply. Nowadays, preservatives are frequently used to keep fish from spoiling. The effects of using chemical preservatives (Sodium benzoate and potassium sorbate) and locally prepared liquid smoke prior to smoking and oven drying of tilapia fish were carried out using standard microbiological methods. In the result obtained across Day 0 to Day 21 of storage, microbial load (THB, TF, TCC and TSC) ranged between 3.25-6.92 $\log_{10}$ cfu/g for the control (Untreated samples) and zero/no count - 5.82 $\log_{10}$ cfu/g for the treated samples, after they samples were subjected to treatments prior to smoking and oven drying the THB count was reduced. Although, there was no significant difference $P<0.05$ between the treatments used. However, the greatest reduction was seen in the oven dried fish samples treated with potassium sorbate (ODPS). The microbiological load count of fish samples subjected to liquid smoke treatment exhibited the highest value after a storage period of 21 days, in contrast to fish samples treated with potassium sorbate, which had the lowest microbial load count after the same storage duration. *Bacillus* sp., *Staphylococcus* sp., *Vibrio* spp., *Micrococcus* sp. and *Klebsiella* sp. were the most predominant bacterial species isolated from the treated samples while the isolated fungi were identified as *Aspergillus* sp., *Saccharomyces* sp., *Penicillium* sp., *Fusarium* sp., and *Rhizopus* sp.. The result obtained proved that the treatments used on the fish samples effectively reduced the microbial count.

Keywords: Sodium benzoate, potassium sorbate

Introduction
Fish is considered to be one of the most nutritious meal options available. The food item contains significant amounts of essential nutrients, including protein and vitamin D. Additionally, it serves as a significant reservoir of omega-3 fatty acids, which play a vital role in the functioning of the human body and cognitive processes. Fish is well recognized as a crucial component of the dietary habits of rural and fishing communities in Nigeria, where it contributes a substantial proportion, up to 75%, of the overall animal protein consumption. From an economic standpoint, fish serves as a crucial source of sustenance and financial support for individuals of both genders. Moreover, fishing has a significant social and cultural significance among riverine communities. According to Rahji and Bada (2010) [21], Nigeria has a yearly fish consumption requirement of approximately 1.5 million tonnes. In order to meet the recommended minimum fish consumption rate of 12.5 kilograms per person per year, as suggested by the Food and Agriculture Organization (FAO), Nigeria needs to bridge the fish supply-demand gap. Currently, the nation imports up to 400,000 tonnes of fish to fulfil this gap. The provision of fish to customers in a timely manner, appropriate shape, and suitable location, while minimizing costs, necessitates the implementation of an efficient marketing strategy.

Fish populations fluctuate annually throughout fishing meccas due to a variety of variables, the most prominent of which are changes in the fish's nutrition and activity level. One of the most popular fish species farmed in Nigeria is the tilapia. This tropical fish matures quickly and often outgrows its kin in size. Tilapia are more suited to freshwater habitats including streams, ponds, and lakes than saltwater habitats. Tilapia is in significant demand in the Nigerian market because of its excellent productivity (Jagdish, 2021) [10].

The necessity to preserve fish parts after harvest arises from the need to prevent spoilage, extend storage life, and minimize the likelihood of food illness. The rapid proliferation of microorganisms and the presence of endogenous enzymes, both of which occur naturally in fish and may potentially be the consequence of contamination, considerably accelerate the deterioration of fresh fish after capture. The decomposition of fish results in the formation of new molecules. When fish spoils, a number of things happen: proteins and lipids break down; the fish's aroma, flavour, and texture change; and the fish's chemical makeup changes. Consequently, there is a pressing need to innovate effective treatment methods for extending the fish's shelf life.

Various preservation methods are used to extend the shelf life of fish, ensuring its suitability for food. These techniques include sun drying, solar drying, freezing, canning, and the application of heat via smoking, among other methods. The practice of smoking is a prevalent and cost-effective technique used for the preservation of fish in Nigeria, Ghana, and other nations in West Africa (Adeyemi *et al*., 2013; Nyarko, 2011) [1, 13]. At lower temperatures, the use of refrigeration and freezing techniques aids in the preservation of fish by inhibiting the growth and activity of microorganisms. Consequently, the fish may be maintained in a state of reduced deterioration for an extended period of time. While some preservation techniques like canning and freezing may be cost-prohibitive in underdeveloped nations, smoking is often suggested as a more accessible approach that does not need advanced equipment or specialised labour (Olayemi *et al*., 2011) [18]. According to a study conducted by Aliya *et al*. (2012) [3], around 25 to 30% of the global fish harvest is used via the utilisation of drying, salting, smoking, or a combination of these preservation methods. Smoking fish facilitates an extended shelf life, improved storage feasibility, and an enhanced flavour profile. The smoking procedure induces a reduction in the moisture content of the fish, making it suitable for preservation and transportation to alternative locations for eating. The act of smoking. The duration of fish preservation by smoking might vary between one week and one month, depending upon the duration of the smoking process. Nevertheless, the act of smoking requires constant vigilance and the use of potentially expensive equipment. Additionally, there are statistical associations that suggest the presence of carcinogenic substances in smoked meals. If adequately prepared and consumed in appropriate quantities, the aforementioned issues may be alleviated.

Various preservation techniques, like as smoking and the use of chemical preservatives, may be employed to delay or prevent microbiological decomposition of fish. The aforementioned substances include sodium benzoate and potassium sorbate. Potassium sorbate is well recognised as an effective preservative against a broad range of bacteria that cause food degradation. Preservatives used in the contemporary food business are recognised for their exceptional safety, efficiency, and versatility. According to Omojowo *et al*. (2009) [17], sorbates exhibit a lack of taste and aroma. Due to their non-toxic nature, these substances find use in a diverse range of culinary items, such as cheese, yoghurt, sour cream, bread, cakes, baking mixes, icing, drinks, margarine, fermented vegetables, fruit goods, salad dressing, smoked and salted fish, and mayonnaise. Researchers have documented the antibacterial efficacy of sorbates against moulds, bacteria, and fungi (Sofos, 2000) [23]. Sodium benzoate has antibacterial characteristics, effectively inhibiting the proliferation of bacteria and mould. Sodium benzoate is used in the preservation of fish due to its bacteriostatic and fungicidal qualities. It significantly extends the duration of fish preservation while maintaining its texture, flavour, and visual qualities.

Fish degradation keeps causing post- harvest losses and a decline in fish supply. Fish that has been contaminated by microorganisms and chemical processes loses part of its nutritional and organoleptic properties, rendering it unfit for human eating. However, there have been attempts to preserve and process fish as well as increase its shelf stability by pretreating fishes before drying them. Different preservative techniques such as liquid smoke application, drying and the use of chemical preservatives like potassium sorbate and sodium benzoate, can be used to postpone or prevent microbial decomposition of fish. Additionally, there is an urgent need for a method that is both effective and affordable and can maintain fish quality for an extended period of time while simultaneously addressing the protection of the fishes from complete spoilage. In contrast to this framework, this research was carried out. The aim of this study is to evaluate the effects of chemical preservatives (Sodium benzoate and potassium sorbate) and liquid smoke on the microbial quality of oven dried and smoked tilapia fishes (*Oreochromis niloticus*) during storage at room temperature.

Materials and Methods

Study area

The study was carried out in Obio/Akpor Local Government Area (LGA), situated within the geographical boundaries of Rivers State. The research region is situated within the approximate geographical coordinates of latitudes 40° 50'08.24" N to 40° 52'20.49" N, and longitudes 70° 02'18.48" E to 70° 06'05.20" E. Obio/Akpor is a Local Government Area located inside the urban centre of Port Harcourt, which is widely acknowledged as a significant economic centre in Nigeria. Furthermore, Port Harcourt has considerable importance as a prominent metropolitan hub situated in the Niger Delta area, notably positioned in the state of Rivers. The administrative centre of Obio/Akpor is situated in the locality of Rumuodomaya. The establishment of the entity occurred on May 3, 1989, as an independent organisation distinct from the Onuoha *et al*. 554 Port Harcourt City Local Government of Rivers State. This development took place during the tenure of President Ibrahim B. Babangida's Military Administration. The Council Area is situated in close proximity to Emohua, Ikwerre, Etche, Oyigbo, Eleme, Okrika, and Port Harcourt Local Government Areas within the state of Rivers. The population of the area mostly consists of individuals belonging to the Ikwerre Ethnic Nationality. However, the urban status of the region and the welcoming nature of its inhabitants contribute to its diverse demographic composition. The Local Government Area is seeing an increase in the number of individuals from various nationalities settling in the region. The Local Government Area has an area of 260 square kilometres and had a population of 464,789 individuals in the 2006 Census, as reported by obioakporembassy.com

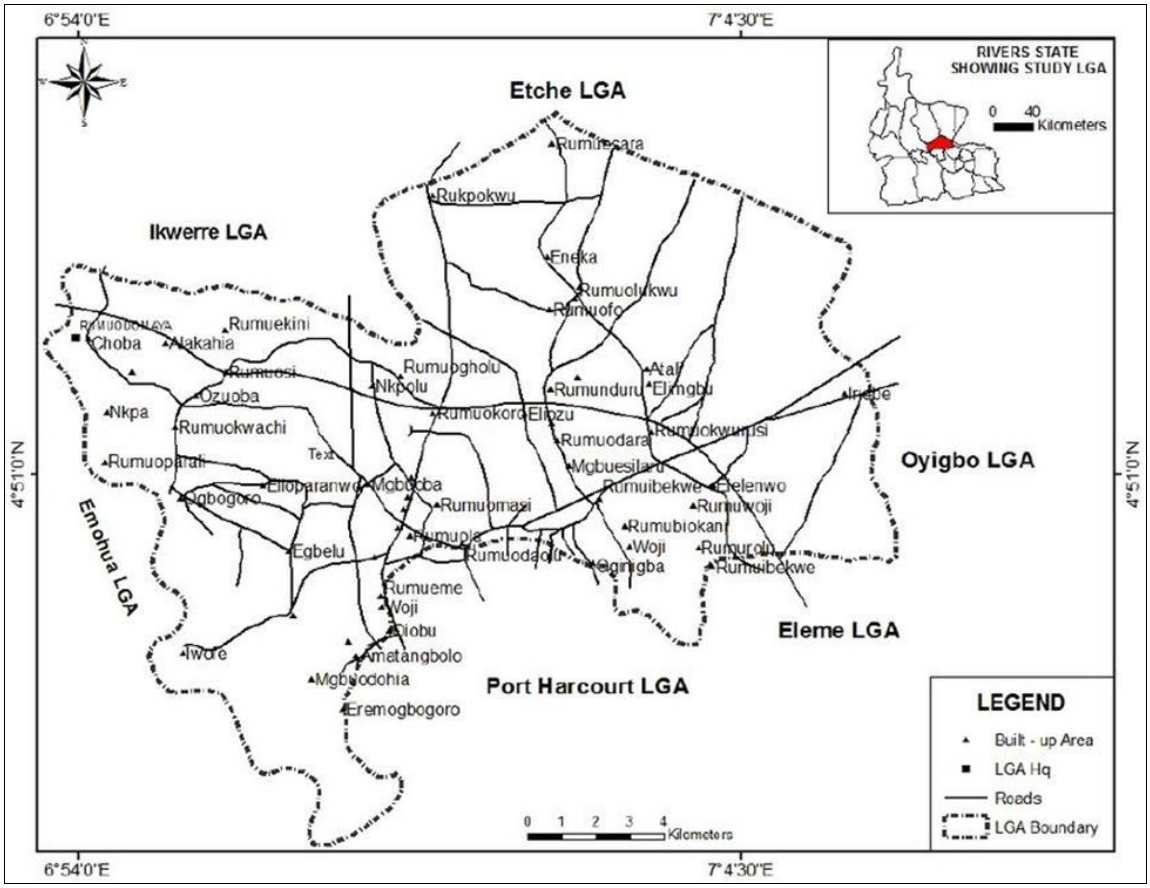

Fig 1: Map showing study area of Obio Akpo Local Government Area, Rivers State, Nigeria (Source: Google)

Preparation of the Liquid smoke

The coconut shell waste was obtained from different coconut vendors within Choba and Ozuoba community and were sundried for three days to reduce excess moisture before been used in preparation of liquid smoke. The liquid smoke was prepared through pyrolysis process according to Budaraga *et al*. (2016) [28] with slight modifications. Equipment used in pyrolysis was a locally improvised smoke condensation equipment that consists of a condenser unit, pipe, distillate bottle, source of heat and other equipment used. The product was then stored in an air container until it was used.

Preparation of the chemical preservatives

The chemical preservatives were prepared according to the method described by Irwin (1976) [7]

Weight/volume percent (% w/v) = weight in g of a solute per 100 ml of solution

To prepare a 0.2% concentration of sodium benzoate, 0.2 g of sodium benzoate was dissolved in a distilled water to make a 100 ml of solution. At the same above condition, 0.2% potassium sorbate was also prepared.

Source of fish samples

Fresh tilapia fishes (*Oreochromis niloticus*) were procured from the Choba and Ozuoba communities, located in the Obio Akpor Local Government Area of Port Harcourt, Rivers State, Nigeria. The fish samples were promptly transported to the laboratory in sterile polythene bags within a time frame of 30 minutes. Subsequently, the fish were euthanized by severing the spinal cord using a sterile knife. Following this, the fish were aseptically eviscerated, washed, and rinsed in sterile water. The fishes were allocated into eight groups, each consisting of five fishes, following the methodology outlined by Omojowo *et al*. (2009) [17], with some adjustments. This experimental design was used specifically for the tilapia species under investigation. Group 1,2 and 3 were treated with 0.2% concentration of sodium benzoate, 0.2% concentration of potassium sorbate for 5 minutes and liquid smoke was applied round the fishes five times for twenty minutes using a clean brush before heating according to (Hesham *et al*., 2021) [11] procedure prior to smoking, Group 4, 5 and 6 were also treated with 0.2% concentration of sodium benzoate, 0.2% concentration of potassium sorbate and liquid smoke before they were an oven dried group 7 and 8 served as a control (untreated samples) which was smoked and oven dried. All they samples were stored in an airtight container and drawn for sampling after 7 days, 14 days and 21 days of storage then subjected to analysis.

Smoking of the fishes

The fish samples were smoked using the hot smoking method, the smoking kiln was locally improvised with blocks of about 0.3 m height which were used to raise the wire mesh to avoid direct contact with fire and smoking was done using heat from coal as a source of heat. Hot smoking was done according to the method described by (Olusegun *et al*., 2013) [19] the fishes were turned at the same time to maintain uniform smoking at an interval of one hour thirty minutes until a uniform brown colour was achieved. The oven dried fishes were put into an oven and dried at 45 °C, 65 °C and 80 °C for two hours.

Storage of the fishes
The fish samples were labeled properly and stored in an airtight container at room temperature of about 30-37 °C and were drawn after seven, fourteen and twenty-one days of storage, then subjected to analysis.

Preparation of media
All analytical procedures are according to the AOAC.

Total Heterotrophic Bacteria Count (THB)
The quantification of heterotrophic bacteria was conducted by using the nutrient agar medium and the pour plate technique, following the methodology outlined by Prescott *et al*. (2005) [20]. A quantity of ten grammes (10 g) of each sample was carefully measured and placed into a conical flask with a capacity of 250 ml. The flask already contained 90ml of sterile normal saline solution. This process resulted in an initial dilution of 10^{-1}. A volume of 1 mL was extracted from the aforementioned dilution and combined with 9 mL of sterile normal saline in a test tube, resulting in a dilution of 10^{-2}. The aforementioned procedure was iterated until a dilution of 10^{-6} was achieved. An aseptic transfer of a 1 mL aliquot from the suitable dilution was performed onto sterile Petri plates. Subsequently, 10 mL of molten sterile agar, cooled to about 45 °C, was aseptically added to the dishes. The mixture was gently stirred and then allowed to harden. The samples were plated in duplicate. The plates were subjected to incubation in an inverted orientation at a temperature of 35±2 ºC for a duration of 24 hours. Following this incubation period, the number of viable colonies present on the plates was determined.

Total Coliform (TCC)
The determination of the total coliform count was conducted using MacConkey Agar and the spread plate technique, as outlined in the methodology given by Prescott *et al*. (2005) [20]. A quantity of ten grammes (10 g) was measured for each of the samples and placed into a 250 ml conical flask. The flask already contained 90 ml of sterile normal saline. This resulted in an initial dilution of 10^{-1}. A volume of 1 mL was extracted from the aforementioned diluted and added to a test tube containing 9 mL of sterile normal saline, resulting in a dilution of 10^{-2}. The aforementioned procedure was iterated until a dilution of 10^{-6} was achieved. A 0.1 ml aliquot of the suitable dilution was aseptically placed onto the surface of pre-dried sterile Petri plates. The sterile bent glass rod was used to evenly distribute the plates, which were afterwards incubated in an inverted posture at a temperature of 35±2 ℃ for a duration of 24 hours. Following this incubation period, the number of viable colonies was determined by counting.

Total *Staphylococcus aureus* counts (TSC)
Pour plate method was employed for the growth of *S. aureus* counts. About 0.1 ml of the serially diluted samples was aseptically plated on Mannitol Salt Agar and incubated inverted at 37 °C for 24 h. The presence of yellow pigment, which did not show haemolytic properties on blood agar indicate *S. aureus* (Ineyougha *et al*., 2015) [8]

Purification of isolates
Each bacteria isolate was further subcultured aseptically from the plates by streak plate method into newly prepared nutrient agar plates using a wire loop and incubated at 37 °C for 24 hours. Furthermore, the bacterial isolates that exhibited growth on Mannitol Salt Agar (MSA) were then subcultured onto a nutrient agar plate. Gramme staining and several biochemical analyses were performed on all pure cultures. The process of sub culturing was used to cultivate fungal isolates. Spores were obtained using a wire loop and afterwards put onto a fresh Potato Dextrose Agar (PDA) plate. The plate was then incubated at a temperature of 37 degrees Celsius for a duration of 5 days.

Characterization and identification of isolates
The bacteria that were kept in isolation were examined in order to evaluate their characteristics related to colony shape, cell micro-morphology, and biochemical properties. The following laboratory tests were performed: The Gram reaction, citrate utilisation test, catalase test, indole test, methyl red test, fermentative utilisation of glucose, indole test, and test for hydrogen sulphide (H2S) generation from Triple Sugar Iron Agar (TSIA). The process of identifying fungal isolates included the examination of macroscopic characteristics, such as shape, colour, spore arrangement, mycelium structure, hyphae structure, and sporangiophore organisation, among others.

Molecular Identification
Bacterial genomic DNA extraction
The genomic DNA was obtained using the Quick-DNA fungal/bacteria Miniprep kit (Zymo Research, Catalogue No. D6005) following the protocol provided by the manufacturer (http://www.zymoresearch.com).

Amplification, sequencing and purification
To enhance the amplification of the 16s target region, we used the primers specified in Table 3.1 and the OneTaq Quick-Load 2X Master Mix (NEB, Catalogue No.M0486). Following the completion of the polymerase chain reaction (PCR), the DNA obtained from the gel was isolated with the Zymoclean Gel DNA Recovery kit (Zymo Research, Catalogue No D4001). The isolated fragments were sequenced using a forward and reverse sequencing kit (Nimagen, Brilliant Dye Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000) and then purified using a purification kit (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit, Catalogue No D4050). The procedures described in Section 1 were conducted using the ABI 3500xl Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific) on the purified fragments obtained from each sample. The.ab1 files generated by the ABI 3500XL Genetic Analyzer were subjected to analysis using BLAST (NCBI) and CLC Bio Main Workbench v7.6.

16S Primers sequences

Name of primer	Target	Sequence (5' to 3')
16S-27F	16S rDNA sequence	AGAGTTTGATCMTGGCTCAG
16S-1492R	16S rDNA sequence	CGGTTACCTTGTTACGACTT

Total Fungi Count (TF)
In order to decrease the bacterial population, a 1% concentration of lactic acid was included into Potato Dextrose Agar (PDA) (Okerentugba & Ezereonye, 2003) [3] prior to quantifying the fungal growth on the culture medium. Prescott *et al*. reported the use of the spread plate

approach. A sterilised glass spreader was used to evenly distribute a 0.1ml aliquot of the appropriate serially diluted samples onto sterile pre-dried Potato Dextrose Agar (PDA) plates. Following a period of 5 days in an incubator maintained at a temperature of 250 degrees Celsius, a measurement of the average number of colonies was conducted and then documented (Douglas & Robinson, 2018) [4].

Statistical analysis

Statistical analyses were conducted with the help of a social science statistical program (SPSS Version 23.1). When detecting significant variations among sample values, a two-way analysis of variance (ANOVA) was utilized. When distinguishing significant means, least significant difference was employed by fisher method.

Results and Discussions

Bacteria group

The total heterotrophic bacteria count (THB), total coliform count (TCC) and total *Staphylococcus* count (TSC), during the 21 days of storage are represented in the figure 1 to figure 4. Respectively. Based on the results obtained across Day 0-21 of the study, the total heterotrophic bacteria count (THB) of the untreated samples (control) ranged from highest 6.92 to the lower 4.78 $\log_{10}$ Cfu/g while the total heterotrophic bacteria count (THB) of the treated samples ranged from highest 5.82 to the lower 4.19 $\log_{10}$ Cfu/g. Oven dried fishes treated with potassium sorbate (ODSB) significantly reduced the total heterotrophic bacteria count when compared to other treatments as seen in the figure. 2. The total coliform count (TCC) of the untreated samples (control) ranged from highest 4.63 to lowest 3.20 $\log_{10}$ Cfu/g, the TCC of the treated samples ranged from highest 3.83 $\log_{10}$ Cfu/g to lowest zero/no count with the highest reduction seen in smoked fishes treated with sodium benzoate (SFSB) and smoked fishes treated with potassium sorbate (SFPS). The total *Staphylococcus* count (TSC) of the untreated samples (control) ranged from highest 3.65 to lowest 3.29 $\log_{10}$ Cfu/g while the total *Staphylococcus* count (TSC) of the treated samples ranged from highest 2.81 to lowest 2.04 $\log_{10}$ Cfu/g oven dried fish samples treated with potassium sorbate significantly reduced the total *Staphylococcus* count compared to other treatments.

Molecular identification

When compared to the NCBI non-redundant nucleotide (nr/nt) database, the acquired 16s rDNA sequence from the isolate yielded an exact match during the mega blast search for highly similar sequences. Isolates' 16S rDNA exhibited a similarity to other species of 96.68%. Plate 1 displays the final product, which is *Staphylococcus sciuri* bacteria that were cultured and grown in isolation.

Fungi isolation

The total fungi count (TF) for the fish samples are represented in Figure .8 and Figure 9. The total fungi count (TF) of the untreated samples (control) ranged from the high 4.73 to the lower 4.48 $\log_{10}$ Cfu/g, while the total fungi (TF) of the treated samples ranged from highest 4.32 to lowest 2.41 $\log_{10}$ Cfu/g. Oven dried fishes treated with potassium sorbate (ODPS) significantly reduced the total fungi count compared to other treatments as seen in the figure 8. Table 4.3 represents the microscopic and macroscopic characteristics of fungi isolated from the study. The fungi isolates were identified as *Aspergillus* sp, *Saccharomyces* sp, *Penicillium* sp, *Fusarium* sp, *Rhizopus* sp.

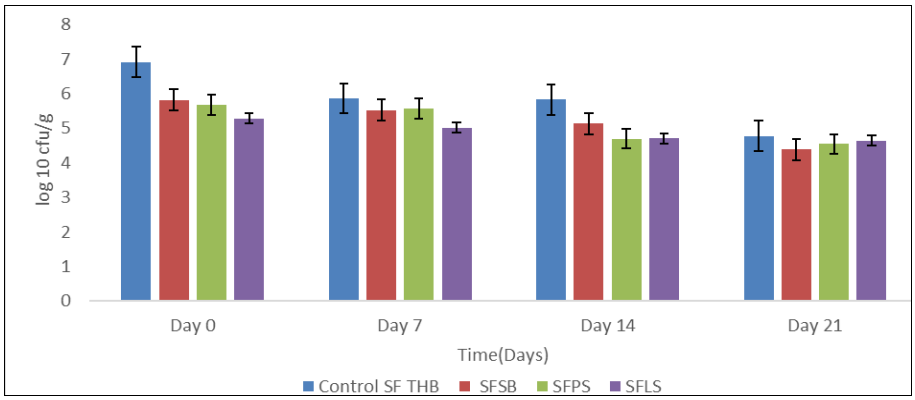

Fig 1: Total Heterotrophic Bacteria count (THB) for Smoked Fish samples Key: SFSB = Smoked fishes treated with sodium benzoate, SFPS = Smoked fishes treated with potassium sorbate, SFLS = Smoked fishes treated with liquid smoke

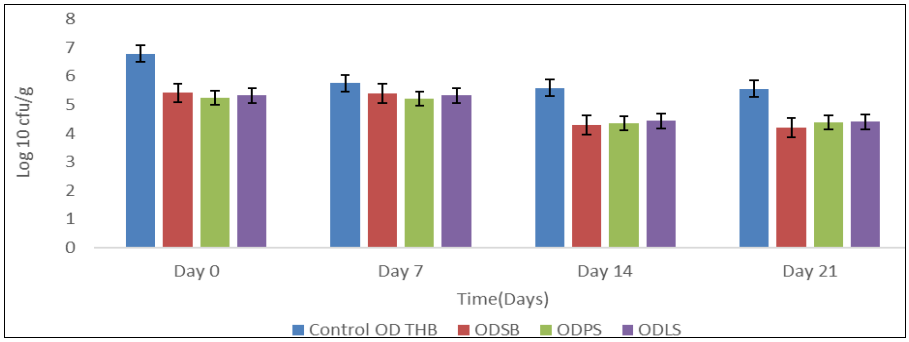

Fig 2: Total Heterotrophic Bacteria count (THB) for Oven dried Fish Key: ODSB = Oven dried fishes treated with sodium benzoate, ODPS = Oven dried fishes treated with potassium sorbate, ODLS = Oven dried fishes treated with liquid smoke

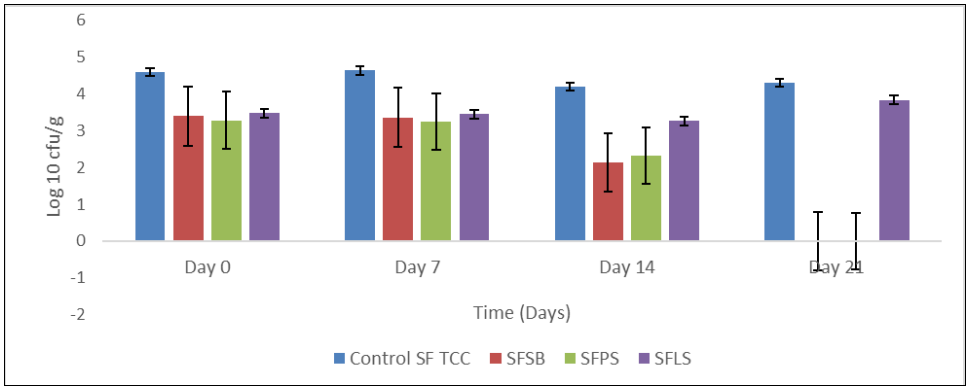

Fig 3: Total Coliform Count (TCC) for Smoked Fish samples, Key: SFSB = Smoked fishes treated with sodium benzoate, SFPS = Smoked fishes treated with potassium sorbate, SFLS = Smoked fishes treated with liquid SMOK

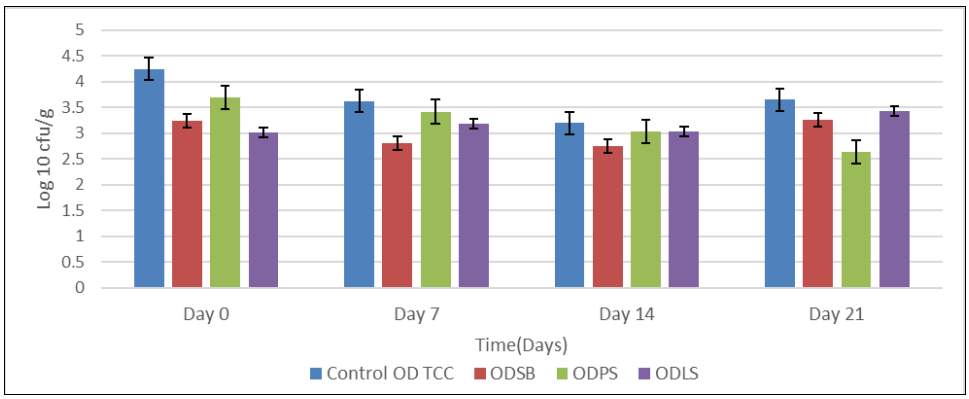

Fig 4: Total Coliform Count (TCC) for oven dried Fish samples, Key: ODSB = Oven dried fishes treated with sodium benzoate, ODPS = Oven dried fishes treated with potassium sorbate, ODLS = Oven dried fishes treated with liquid smoke

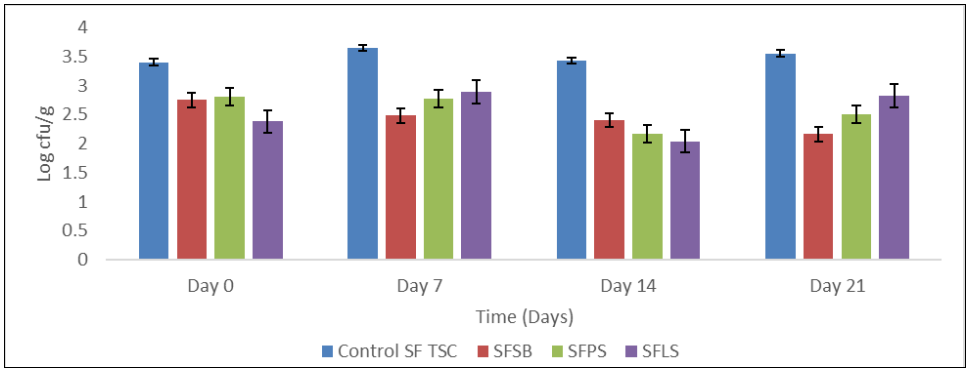

Fig 5: Total *Staphylococcus* Count (TSC) for Smoked Fish samples, Key: SFSB = Smoked fishes treated with sodium benzoate, SFPS = Smoked fishes treated with potassium sorbate, SFLS = Smoked fishes treated with liquid smoke

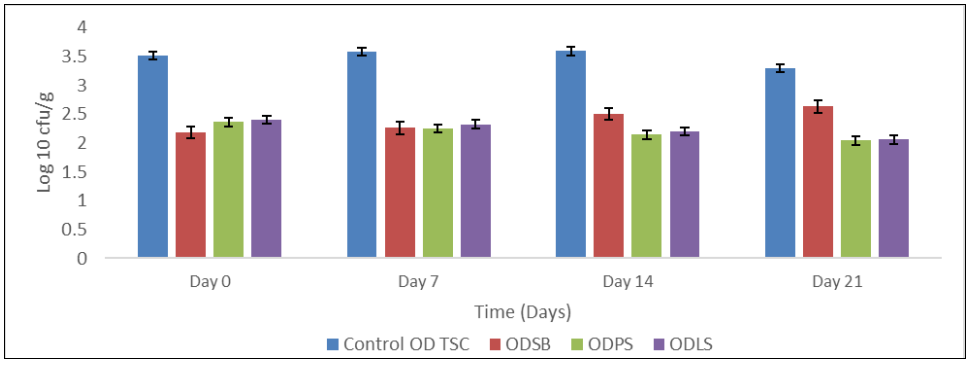

Fig 6: Total *Staphylococcus* Count (TSC) for Oven dried Fish samples, Key: ODSB = Oven dried fishes treated with sodium benzoate, ODPS = Oven dried fishes treated with potassium sorbate, ODLS = Oven dried fishes treated with liquid

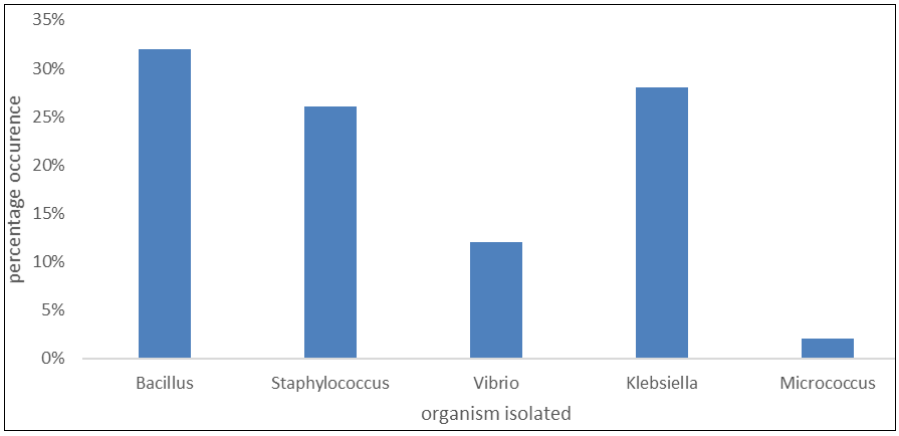

Fig 7: The percentage occurrence of Bacteria isolated from treated samples

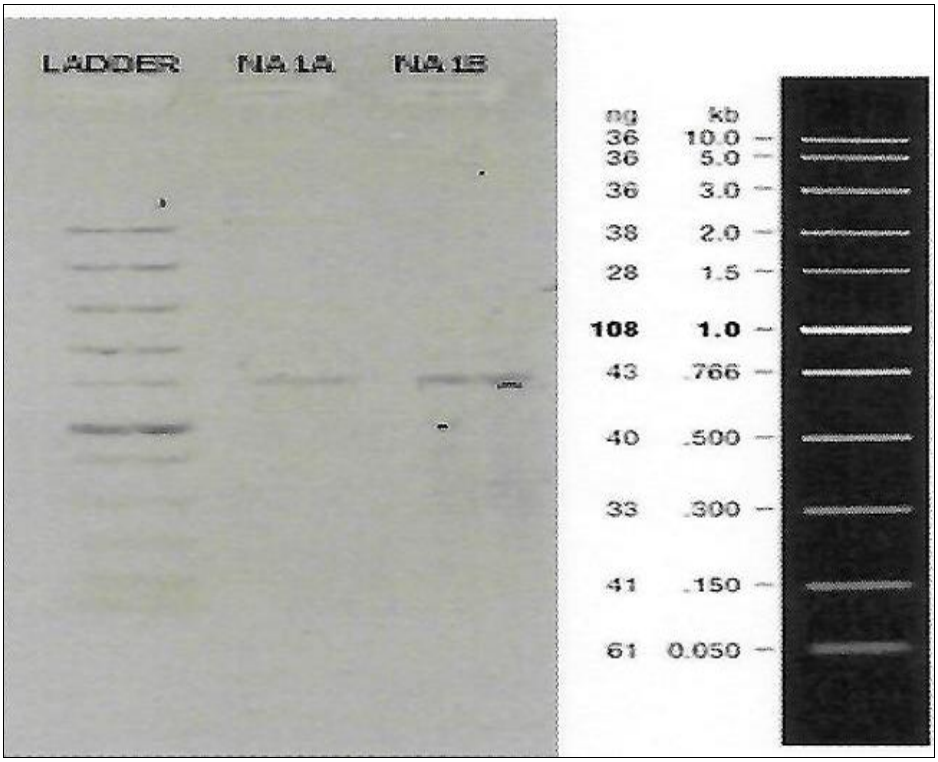

Plate 1: Photographic image of an agarose gel indicating the implication of the 16S target region in *Staphylococcus sciuri*

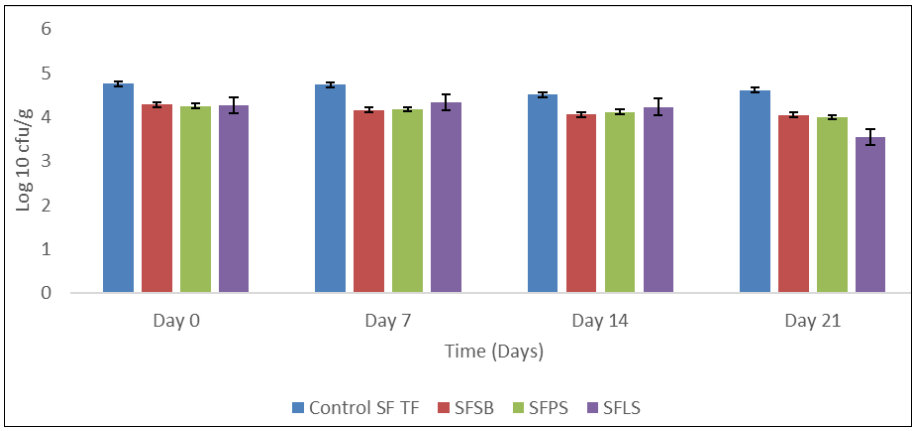

Fig 8: Total Fungi Count (TF) for Smoked Fish samples
Key: SFSB = Smoked fishes treated with sodium benzoate, SFPS = Smoked fishes treated with potassium sorbate, SFLS = Smoked fishes treated with liquid smoke

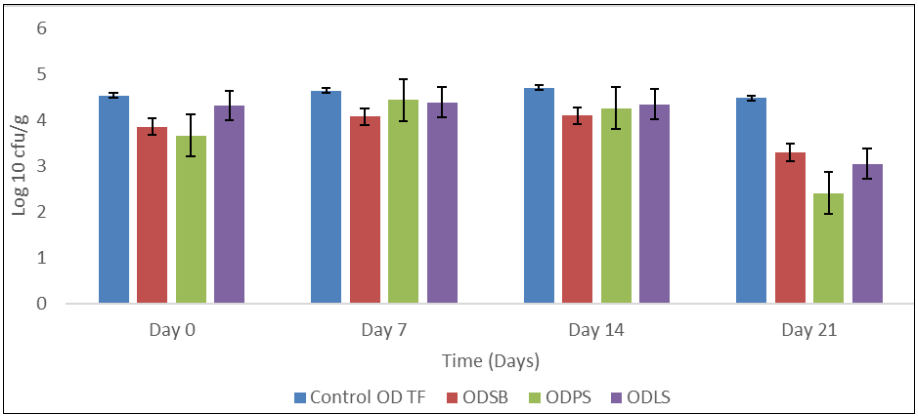

Fig 9: Total Fungi Count (TF) for oven dried Fish samples, Key: ODSB = Oven dried fishes treated with sodium benzoate, ODPS = Oven dried fishes treated with potassium sorbate, ODLS = Oven dried fishes treated with liquid smoke

Discussion

Food items containing protein, like fish, are susceptible to bacterial contamination from the surrounding environment. Microbial growth in these products can lead to unfavorable physical and chemical alterations, rendering them unsuitable for consumption due to potential health hazards. Hence, it was crucial to assess the bacterial count in fish samples to ascertain their suitability for consumption. (Swastawati *et al*., 2022) [24].

The total heterotrophic bacteria count (THB) as determined in this study revealed that the untreated (control) fish samples were relatively high 6.92 $\log_{10}$ cfu/g to 4.78 $\log_{10}$ cfu/g, but after they samples were subjected to treatments prior to smoking and oven drying the THB count was reduced. Although, there was no significant difference $p<0.05$ between the treatments used. However, the greatest reduction was seen in the oven dried fish samples treated with potassium sorbate (ODPS). It maintained a low level throughout the 21 days of storage. Ineyougha *et al*. (2015) [8] recorded a higher range of total heterotrophic bacteria count (THB) 6.384 to 6.608 $\log_{10}$ cfu/g from smoked *Trachurus*. The total coliform count (TCC) dropped from 4.63 $\log_{10}$ cfu/g to a range of 3.83 $\log_{10}$ cfu/g to no count/zero count at the end of the 21 days of storage, this is similar to the studies of Olusegun & Jacob (2013) [19] during their study, the recorded that the total coliform count reduced from 4.2 $\times 10^4$ to a range of 3.3 $\times 10^4$ - 3.8 $\times 10^4$ for *O. niloticus* at the end of 12 weeks. Coliform bacteria serve as reliable indicators for assessing the cleanliness of food and water. They are described as rod-shaped, gram-negative bacteria that do not form spores and have the ability to ferment lactose, generating acid and gas when kept at temperatures between 35 to 37 degrees Celsius for up to 48 hours. Coliforms typically don't result in severe illnesses. Interestingly, their presence often signals the potential existence of other harmful pathogens originating from fecal matter. (Sivashanthini *et al*., 2012) [25]. The total *Staphylococcus* count (TSC) of the untreated (control) fish samples ranged from 3.65 $\log_{10}$ cfu/g to 3.29 $\log_{10}$ cfu/g while the samples treated with chemical preservatives (sodium benzoate and potassium sorbate) and liquid smoke prior to smoking recorded oven drying ranged a 2.81 $\log_{10}$ cfu/g to 2.04 $\log_{10}$ cfu/g for the 21 days which did not exceed the acceptable limit for total *Staphylococcus* count by International Commission on Microbiology Specifications for seafood (ICMSF, 1998) [9] which is below 10^3 (3 log cfu/g). The high *Staphylococcus* count recorded in the untreated (control) samples might be attributed to post processing contamination. The total *Staphylococcus* count is similar to the findings of Ineyougha *et al*. (2015) [8] whose result indicated TSC values of 2.70 $\log_{10}$ cfu/g - 1.68 $\log_{10}$ cfu/g. The application of liquid smoke on the fish samples also decreased the microbial load Total heterotrophic bacteria (THB), Total fungi (TF), Total coliform count (TCC) and Total *Staphylococcus* count (TSC) compared to the untreated samples (control) this indicates that the liquid smoke acted as an antibacterial agent (Swastawati *et al*., 2022) [24]. According to ICMSF (1996) [9] the maximum recommended bacterial count for good quality fish products is 5.0 x 10^5 (5.0 cfu/g) and the maximum for marginally acceptable quality products is 10^7 (7 log cfu/g) (Saludeen & Osibona, 2018) the result obtained in this study was below the acceptable limit. *Bacillus sp, Staphylococcus* sp, *Vibro* sp, *Micrococcus* sp and *Klebsiella* sp were the most predominant bacterial species isolated from the treated samples, but they were negative for *E.coli* and *Streptococcus* which was similar to the findings of Omojowo *et al*. (2009) [17] with *Micrococcus* sp having the highest occurrence of 32%. The presence of *Staphylococcus, Bacillus, Klebsiella* in this study might be as a result of the moisture content of the product and also increase in temperature that favors the growth of the organism. The isolation of *Bacillus* sp and *Staphylococcus* sp is an indication of poor handling or cross contamination of fish products since the two organisms have been indicted in food poisoning (Dike-Ndudim *et al*., 2014) [5]. *Klebsiella* which was also identified is among the most common gram-negative bacteria. Infections such as pneumonia are caused by *Klebsiella pneumoniae* and respiratory tract diseases resulting in having low immunity. *Micrococcus* isolated can possibly cause arthritis and bacteremia in humans (Wharton *et al*., 1986) [26]. While *Vibrio* sp a gram-negative bacterium found in fish and shellfish living in saltwater and in rivers causes vibriosis, an intestinal disease. According to Herman *et al*. (2011) [12] The *Staphylococcus* bacterial group stands as a prevalent source of human illnesses and forms part of the regular microorganisms found on the human skin and mucous membranes, typically without causing any disease. Akpabio *et al*. (2018) [2] isolated *Bacillus* sp, *Staphylococcus* sp and *Micrococcus* sp from smoked fishes while Moshood *et al*. (2012) [14] isolated *Bacillus* sp, *Staphylococcus* sp, *Proteus*, *Klebsiella*, *Salmonella* and *Streptococcus* which are similar to the bacteria isolated in this study except for the absence of *Salmonella* and *Streptococcus* of this study.

Through the process of DNA sequencing, researchers successfully ascertained that the isolated bacterium had familial characteristics with *Staphylococcus sciuri*. The total fungi count (TF) of the oven dried fish samples treated with potassium sorbate had the lowest reduction of 2.41 log_{10}cfu/g. this is similar to the studies reported by (Omojowo *et al*., 2009) [17] where the samples treated with potassium sorbate showed maximum reduction in microbial load. The isolated fungi were identified as *Aspergillus* sp, *Saccharomyces* sp, *Penicillium* sp, *Fusarium* sp, and *Rhizopus* sp. The occurrence of fungi may be due to the difference in the chemical composition of the fish samples and to which different moulds react differently of the species. (Olajuyigbe *et al*., 2017) [16] isolated *Aspergillus, Penicillium, Fusarium, Rhizopus, Mucor, Neurospora* and *Trichoderma* fungi species from any fishery product which is similar to the fungi isolated in this study, except for *Mucor, Neurospora* and *Trichoderma*. These isolated fungi are potential pathogens and are likely to have mycotoxins (Olajuyigbe *et al*., 2017) [16]. In conclusion, the microbial load analysis conducted in this study indicate that the utilisation of chemical preservatives, namely sodium benzoate and potassium sorbate, as well as liquid smoke for the treatment of fish samples prior to the processes of oven drying and smoking, resulted in a significant reduction in the quantities of total heterotrophic bacteria (THB), total staphylococci (TSC), total coliforms (TCC), and total fungi (TFC) observed in the smoked and dried fish samples, in comparison to the control samples. Based on our analysis at the significance level of p=0.05, we observed no statistically significant difference between the various treatments. The microbiological load count of fish samples subjected to liquid smoke treatment exhibited the highest value after a storage period of 21 days, in contrast to fish samples treated with potassium sorbate, which had the lowest microbial load count after the same storage duration. The bacterial strains that were isolated in this study consist of *Bacillus* sp., *Staphylococcus* sp., *Vibrio* sp., *Micrococcus* sp., and *Klebsiella* sp. Similarly, the fungal strains that were identified include *Aspergillus* sp., *Saccharomyces* sp., *Penicillium* sp., *Fusarium* sp., and *Rhizopus sp.* Through the process of DNA sequencing, researchers successfully ascertained that the isolated bacterium had familial characteristics with *Staphylococcus sciuri*. This study assessed the impact of chemical preservatives (sodium benzoate and potassium sorbate) and liquid smoke on the oven dried and smoked fish samples for a storage period of 21 days. The result obtained proved that the treatments used on the fish samples effectively reduced the microbial count.

Recommendations

- The combined effect of pretreating fish before smoking and oven drying in fish processing should be employed to significantly increase the effectiveness of each technique.
- This study proved that the use of liquid smoke should be considered as an easy and simple technology in fulfilling the need of a natural treatment.

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