

EFFICACY OF PINEAPPLE CROWN ON BACTERIAL SAFETY OF *Clarias gariepinus* AND *Malapterurus electricus* SMOKED FILLETS

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ABSTRACT

The study evaluated the efficacy of *Ananas comosus* crown as a natural preservative for improving the bacterial safety of smoked *Clarias gariepinus* and *Malapterurus electricus* fillets. Fresh samples of both fish species were obtained from Kalgwai Barrage Dam in Jigawa State, Nigeria, filleted, and subjected to seven treatments: control (no preservative), 5%, 10%, and 15% each for salt solution and *A. comosus* crown solution. The treated fillets were smoked and stored at ambient temperature for four months. Phytochemical analysis of the pineapple crown revealed the presence of saponins, flavonoids, tannins, alkaloids, and glycosides, with saponins occurring in the highest concentration. The bacterial loads of raw fillets were 7.07×10^4 cfu/g for *C. gariepinus* and 7.90×10^4 cfu/g for *M. electricus*, with no significant difference between the species ($P > 0.05$). After the smoking, samples treated with salt and *A. comosus* crown solution showed significantly lower bacterial loads than the control ($P < 0.05$). Higher concentrations of both treatments were more effective in reducing microbial counts. During the four-month storage period, bacterial loads increased gradually in all treatments. The predominant bacterial isolates identified were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella* spp., *Escherichia coli*, and *Salmonella* spp., with *S. aureus* being the most prevalent. The results indicate that pineapple crown possess antimicrobial properties and can serve as an effective natural preservative for improving the microbial quality of smoked fish fillets.

Keywords: *Ananas comosus* crown, fish processing, fish smoking, natural preservative, phytochemicals.

INTRODUCTION

Fish is an affordable and widely available sources of high-quality protein for human consumption (Olugbojo and Ayoola, 2015). It is a major component of the human diet for centuries and continues to play a significant role in the nutrition of many populations. Fish is valued for its high biological quality, characterized by efficient protein utilization in the body, low cholesterol content, and the presence of essential amino acids.

Zulema (2014) recommended regular fish consumption due to its role in preventing cardiovascular diseases and other forms of ailment. Ravichan-dran *et al.* (2011) reported that fish serves as a rich source of antimicrobial peptides that help protect the body against harmful human pathogens. Because fish contains all essential amino acids, it is classified as a complete protein, making its inclusion in the human diet important (Pawar and Sonawane, 2013). Heliene (2016) further stated that fish is rich in polyunsaturated fatty acids, which support health promotion and maintenance, as well as essential minerals such as calcium, phosphorus, sodium, potassium, and magnesium. Nigeria ranks among the world's major fish consuming countries, with an annual consumption exceeding 1.5 million tons (Banyigyi *et al.*, 2023). In Nigeria, the demand for fish protein continues to rise due to its affordability and comparatively high nutritional value (Omoniyi and Ojelade, 2017).

However, fish is highly perishable in nature (Ahmed, 2020), and significant quality losses may occur before

consumption if it is not properly handled, processed, and stored. Fresh fish begins to deteriorate rapidly after harvest due to enzymatic activity and microbial action (Tavares *et al.*, 2021). To minimize spoilage, different preservation and processing techniques are used, including smoking, drying, salting, frying, fermenting, and combinations of these methods (Adeyeye *et al.*, 2016).

Fish obtained from temperate and tropical regions, including freshwater and marine environments, harbour diverse spoilage bacteria that serve as the primary agents responsible for the deterioration of aquatic food products (Laorenza *et al.*, 2022; Prasai *et al.*, 2022). When these microorganisms are not effectively controlled, they continue to degrade the fish until it becomes completely spoiled, posing a major challenge to all stakeholders involved in fresh fish production (Mir *et al.*, 2022). Consumption of fish and shellfish can result in disease through infection or intoxication. Some of these illnesses have been linked to pathogenic microorganisms that exhibit resistance to commonly used antibiotics (Adebayo-Tayo *et al.*, 2012). Globally, microbial activity alone accounts for approximately 30% of fish spoilage after landing (Bataringaya, 2007).

Ananas comosus (Pineapple) crown, a waste product generated during pineapple processing, has been reported to possess antimicrobial and antioxidant properties (Moreira, 2021). The crown is rich in bioactive constituents such as bromelain, flavonoids, and phenolic

acids, all of which have been shown to have antimicrobial and antioxidant activities. Despite its potential, it is currently underutilized and is commonly discarded as processing waste. Utilizing this waste product as a natural preservative could promote sustainable and environmentally friendly practices in the fish industry. Therefore, this study was conducted to investigate the efficacy of *A. comosus* crown as a natural preservative for *Malapterurus electricus* and *Clarias gariepinus* fillets. The study sought to evaluate the antimicrobial and antioxidant activities of *Ananas comosus* crown and its effects on the quality of the fish fillets, to provide a sustainable alternative to synthetic preservatives, and reduce waste generation.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at the processing unit, Department of Fisheries and Aquaculture, Faculty of Agriculture, Federal University Dutse, Jigawa State, Nigeria (latitude 11°42' North, longitude 9°22' East), situated in the Sudan Savannah agro-ecological zone of Nigeria.

Source of Experimental Fish

One hundred and twenty fresh *Malapterurus electricus* of average weight ranges of 9 – 507g and average standard length range of 6 – 26cm, and fifty fresh *Clarias gariepinus* of average weight ranges of 55 – 2,000g and average standard length range of 17 – 53cm were purchased from the local fishermen in Kalgwai barrage dam, Auyo Local Government, Jigawa state, and transported to the Department of Fisheries and Aquaculture laboratory, Faculty of Agriculture, Federal University Dutse in a plastic container containing ice for the experiment

Source of *Ananas comosus* Crown

Fresh pineapple crowns were obtained from the people selling the pineapple in Dutse market. The crowns were washed thoroughly with clean water to remove any dirt or debris. The pineapple crowns were dried under shade to reduce the moisture content. The dried pineapple crowns were ground into a fine powder using a mortar and pestle. 5g, 10g, and 15g of the powdered crown were weighed and mixed with 100 mL of mineral water to create 5%, 10%, and 15% solutions, respectively and stirred well (Yanar *et al.*, 2006). The mixture was allowed to steep for 5 hours and strained using a cheesecloth to obtain the pineapple crown solution.

Experimental Design

The experiment consisted of seven treatments. Treatment 1 served as a baseline and did not include any preservative. Salt solution and *A. comosus* were evaluated at three different concentration levels (5%, 10%, and 15%) to determine the optimal concentration for each solution. All treatments were replicated three (3) times in a completely randomized design.

Smoking Treatment

The fish samples were filleted with knife on a cutting

board and washed thoroughly in a clean water. The fillets from each fish species were divided into seven treatment groups, where Treatment 1 serves as a control and did not contain any preservative. Treatment 2, 3 & 4 were brined in varying concentrations of salt solutions at 5%, 10%, and 15% respectively for 10-15 minutes (Yanar *et al.*, 2006; Iwanegbe *et al.*, 2016), followed by a 30-minute water draining. Treatment 5, 6, & 7 were soaked in different concentrations of *A. comosus* crown solution at 5%, 10%, and 15% respectively for 10-15 minutes, followed by a 30-minute water draining. The fillets were arranged at the middle of the wire gauze within the smoking kiln for each of the treatments and smoked with a gentle flame until it is smoked. The fillets were turned regularly at 10 – 15 minutes' intervals to prevent it from charring until dried to a constant weight.

Determination of Phytochemicals Present in *Ananas comosus* Crown

The phytochemical analysis of *Ananas comosus* crown was carried out at the Department of Biochemistry Laboratory, Faculty of Science, Federal University Dutse. The following parameters were determined following the method described by Yadav (2011) and Harbourne (1998): tannins, glycosides, saponins, flavonoids, and alkaloids.

Determination of Bacterial Load of Raw and Smoked *Clarias gariepinus* and *Malapterurus electricus* Fillets

The determination of bacterial load of raw and freshly smoked *Clarias gariepinus* and *Malapterurus electricus* fillets was conducted at the Department of Biochemistry Laboratory, Faculty of Science, Federal University Dutse. The analysis involved the determination of various microbiological parameters, including total viable bacterial counts, identification of specific bacterial species, and enumeration of bacterial load in the fillet samples according to Cheesbrough (2000) and James (2001), following the microbiological procedures recommended in the International Commission on Microbiological Specification for Foods (ICMSF, 1986) and Compendium of Methods for Microbiological Examination of Foods (1992).

Preparation of Culture Media

All culture media used were prepared following the manufacturer's instructions

Enumeration of Aerobic Mesophilic Bacteria

The serial dilution method, as described by the American Public Health Association (APHA, 1992), was used for enumerating aerobic mesophilic bacteria. 11 g of fish sample was mixed with 99 ml of 0.1% peptone water and thoroughly shaken to produce a homogenate, giving a 10^{-1} dilution. 1 ml of the 10^{-1} dilution was transferred into 9 ml of 0.1% peptone water to obtain a 10^{-2} dilution. This procedure was repeated up to 10^{-3} . The dilution bottles were agitated to resuspend any settled material. 1 ml of each dilution was pipetted into corresponding Petri dishes in duplicate. About 15 ml of nutrient agar, cooled to 45°C, was poured into each plate. The sample and agar were mixed by gently rotating the plates on a flat surface and allowed to solidify. Plates were inverted and incubated at



35°C for 48 hours. Plates containing 30–300 colonies were selected for counting. The colony count was multiplied by the dilution factor to obtain the number of bacterial colony forming units per gram (cfu/g) of fish sample.

The formula used: $N = n/vd$

Where: N = number of bacterial colonies per gram of sample, n = number of colonies counted, v = volume of inoculum used, d = dilution factor

This procedure was repeated for all fish samples.

Isolation and Identification of Bacteria from Fish Samples

Isolation

The colonies developing on the plates were observed for colour, size, shape, surface elevation, and margin. Representative colonies of the various morphological types were selected and transferred to freshly prepared, sterilized, and solidified nutrient agar. The inoculated plates were incubated at 35°C for 24 hours to obtain pure cultures of the organisms (APHA, 1992).

Isolation of *Escherichia coli* from Samples

A portion of each isolate was streaked onto previously prepared MacConkey agar using a 3 mm inoculating loop. The plates were incubated at 44°C for 48 hours. Colonies that appeared as small, shiny, pinkish colonies were identified as *Escherichia coli*.

Isolation of *Staphylococcus aureus* and *Staphylococcus epidermidis*

A portion of each of the Isolate was streaked onto the surface of previously dried duplicate plate of Mannitol salt agar. The plates were incubated at 35°C for 48 hours. Opaque Colonies, 1-2mm in diameter appearing yellow and light pink on Mannitol salt agar were primarily identified as *Staphylococcus aureus* and *Staphylococcus epidermidis* respectively.

Isolation of *Pseudomonas aeruginosa* and *Klebsiella spp.*

A portion of each isolate was streaked onto the surface of previously dried duplicate plate of MacConkey agar. The plates were incubated at 37°C for 48 hours. Colonies which had large slimy pink appearance were primarily identified as *Pseudomonas aeruginosa*, while Large mucoid pink colonies on MacConkey agar were primarily identified as *Klebsiella spp.*

Isolation of *Salmonella spp.*

A portion of each isolate was streaked onto the surface of

previously dried duplicate plate of selective media plates of *Salmonella shigella* agar for 24hrs at 35°C. Colonies that appeared uncolored to pale pink, opaque, transparent or translucent as well as those that appeared black centered was countered as *Salmonella*.

Determination of the Bacterial Quality of Smoked *Clarias gariepinus* and *Malapterurus electricus* Fillets

The smoked fish fillets of *Clarias gariepinus* and *Malapterurus electricus* was packaged in properly labeled paper bags to prevent contamination. The paper bags were stored in a cool, dry place at ambient temperature for a period of four months. Room temperature within the storage facility was measured in the morning, by noon and in the evening bi-monthly. Monthly samples (at 1st, 2nd, 3rd, and 4th months) were taken to the Department of Biochemistry Laboratory, Faculty of Science, Federal University Dutse for bacterial analysis to assess the presence and growth of bacterial load, following the microbiological procedures recommended in the International Commission on Microbiological Specification for Foods (ICMSF, 1986) and Compendium of Methods for Microbiological Examination of Foods (1992).

Data Analysis

Descriptive statistics was used to run the result of phytochemical analyses. T-test was used to test for significance difference among treatment means for raw bacterial analyses. The data obtained for the bacterial load were subjected to two-way analysis of variance (ANOVA) using Genstat software to test for significance difference among the treatment means at $P < 0.05$, followed by Duncan's multiple range test to separate the significance difference between the treatments means.

RESULTS

Phytochemical Composition of *Ananas comosus* Crown

The quantitative phytochemical composition of *Ananas comosus* crown is presented in Table 1. Saponins were the most abundant phytochemical component (2.05 ± 0.07), followed by flavonoids (1.83 ± 0.06), tannins (1.41 ± 0.02), and alkaloids (1.05 ± 0.06). Glycosides were detected in the lowest concentration (0.22 ± 0.00). These results indicate that the crown of *A. comosus* contains high quantities of secondary metabolites that may contribute to its preservative and medicinal properties.

Table 1: Quantitative phytochemical composition of *Ananas comosus* crown

PHYTOCHEMICALS				
Tannins	Glycosides	Saponins	Flavanoids	Alkaloids
1.41±0.02	0.22±0.00	2.05±0.07	1.83±0.06	1.05±0.06

Bacterial Load of Raw *Clarias gariepinus* and *Malapterurus electricus* Fillets

The bacterial load of raw *Clarias gariepinus* and *Malapterurus electricus* fillets is presented in Table 2. *C. gariepinus* recorded a bacterial count of 7.07×10^4 cfu/g,

while *M. electricus* recorded a slightly higher value of 7.90×10^4 cfu/g. Statistical analysis showed no significant difference ($P > 0.05$) between the two species. This indicates that the microbial loads of both fish species were comparable in their raw state.

Table 2: Bacterial load of raw *Clarias gariepinus* and *Malapterurus electricus* fillets

Treatments	Bacterial load (cfu/g).
<i>C. gariepinus</i>	$7.07 \pm 0.15 \times 10^4$
<i>M. electricus</i>	$7.90 \pm 0.60 \times 10^4$
P - Value	0.230

Means are presented. Means without * along the column do not vary significantly ($P > 0.05$).

Effect of different Concentrations of Salt and *Ananas comosus* Crown Solution on the Bacterial Load of Smoked *Clarias gariepinus* and *Malapterurus electricus* Fillets.

Table 3 shows the effect of different concentration of salt and *Ananas comosus* crown solution on the bacterial load of smoked *Clarias gariepinus* and *Malapterurus electricus* fillets. For *Clarias gariepinus*, the control

group recorded the highest bacterial load (12.87×10^4 cfu/g), followed closely by 5% salt (11.47×10^4) and 5% *A. comosus* (11.23×10^4). The lowest bacterial load was observed in 10% *A. comosus* (9.76×10^4), which was significantly lower ($p < 0.05$) than the control and salt treatments. *M. electricus* fillets followed the same trend, with the highest bacterial load in the control (12.35×10^4) and the lowest in 15% salt (9.83×10^4).

Table 3: Effect of different Concentrations of Salt and *Ananas comosus* Crown Solution on Bacterial Load of Freshly Smoked *Clarias gariepinus* and *Malapterurus electricus* Fillets.

Treatments	BACTERIAL LOAD (cfu/g)	
	<i>C. gariepinus</i>	<i>M. electricus</i>
(T1) Control	12.87×10^4 a	12.35×10^4 a
(T2) 5% salt	11.47×10^4 b	10.74×10^4 bc
(T3) 10% salt	10.61×10^4 c	10.33×10^4 d
(T4) 15% salt	9.99×10^4 de	9.83×10^4 e
(T5) 5% <i>A. comosus</i>	11.23×10^4 b	11.06×10^4 b
(T6) 10% <i>A. comosus</i>	9.76×10^4 e	10.56×10^4 cd
(T7) 15% <i>A. comosus</i>	10.51×10^4 cd	10.79×10^4 bc
P - Value	0.001	0.001
SE+	0.1945	0.1307
MONTHS		
Feb	4.93×10^4 e	4.22×10^4 e
Mar	7.38×10^4 d	7.04×10^4 d
Apr	9.64×10^4 c	9.6×10^4 c
May	13.96×10^4 b	13.07×10^4 b
Jun	18.71×10^4 a	20.07×10^4 a
P - Value	0.001	0.001
SE+	0.1644	0.1104

Means followed by the same letter within column are not significantly different at 5% level of probability using Duncan Multiple Range Test (DMRT)

Across storage months, bacterial load increased significantly ($p < 0.05$) from February (4.93×10^4 and 4.22×10^4) to June (18.71×10^4 and 20.07×10^4) in *Clarias gariepinus* and *Malapterurus electricus* respectively. This indicates that storage duration and environmental conditions strongly influenced microbial proliferation.

Prevalence of Bacterial Isolates of Freshly Smoked *Clarias gariepinus* Fillets Treated with Different Concentration of Salt and *Ananas comosus* Crown Solution

The bacterial isolates detected from the smoked *Clarias*

gariepinus fillets are presented in Table 4. Across all treatments, six groups of bacteria were identified: *Klebsiella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Salmonella* spp. The highest percentage of occurrence was recorded for *S. aureus* (41.39%), followed by *S. epidermidis* (22.66%) and *P. aeruginosa* (15.79%). *Klebsiella* spp. (13.02%) and *E. coli* (5.00%) were less frequent, while *Salmonella* spp. accounted for the lowest prevalence (2.14%). Among the treatments, samples treated with salt reduced bacterial prevalence, with 15% salt showing the lowest occurrence. Treatment with 5% *A.*



comosus crown solution also reduced bacterial prevalence compared to 15% and 10%

Table 4: Prevalence of Bacterial isolates of freshly smoked *Clarias gariepinus* fillets treated with different concentration of salt and *Ananas comosus* crown solution.

Treatment	Bacterial Isolates					
	<i>klebsiella spp</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>S. epidermidis</i>	<i>Salmonella spp</i>
Control	17	23	32	19	18	1
5% salt	30	8	102	47	46	7
10% salt	29	5	98	36	57	3
15% salt	9	2	44	12	19	1
5% <i>A. comosus</i>	7	5	42	17	16	2
10% <i>A. comosus</i>	41	7	79	28	69	7
15% <i>A. comosus</i>	13	6	67	18	29	3
Total	146	56	464	177	254	24
% of occurrence	13.02	5.00	41.39	15.79	22.66	2.14

Prevalence of Bacterial Isolates of Freshly Smoked *Malapterurus electricus* Fillets Treated with Different Concentration of Salt and *Ananas comosus* Crown Solution

The prevalence of bacterial isolates in smoked *Malapterurus electricus* fillets is shown in Table 5. Six bacterial groups were identified: *Klebsiella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Salmonella* spp. *S. aureus* was the most predominant isolate with the highest frequency (39.67%), followed by *S. epidermidis* (19.68%), *Klebsiella* spp. (15.93%), and *Pseudomonas aeruginosa* (15.93%). *E. coli* accounted for 6.01% of total

isolates, while *Salmonella* spp. recorded the lowest prevalence (2.78%).

Samples treated with salt solution reduced bacterial prevalence, with 15% and 10% salt performing better than 5%. *A. comosus* crown solution showed strong antibacterial effects, especially at 5% and 10%, where significant reductions were observed for *Klebsiella* spp., *E. coli*, and *S. aureus*. However, at 15% *A. comosus* crown solution, *E. coli* (44) and *S. aureus* (110) were higher compared to the lower solution levels, suggesting that excessive concentration may not necessarily enhance antimicrobial action.

Table 5: Prevalence of Bacterial isolates of freshly smoked *Malapterurus electricus* fillets treated with different concentration of salt and *Ananas comosus* crown solution.

Treatment	Bacterial Isolates				
	<i>Klebsiella spp</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella spp</i>
Control	37	7	88	39	6
5% salt	46	11	92	51	5
10% salt	28	4	72	32	3
15% salt	36	6	80	27	2
5% <i>A. comosus</i>	7	5	19	6	1
10% <i>A. comosus</i>	39	3	67	29	6
15% <i>A. comosus</i>	19	44	110	28	14
Total	212	80	528	212	37
% of occurrence	15.93	6.01	39.67	15.93	2.78

DISCUSSION

Phytochemical Composition of *Ananas comosus* Crown

The phytochemical compositions of *Ananas comosus* crown in Table 1 revealed the presence of bioactive compounds including saponins, flavonoids, tannins, alkaloids, and glycosides, with saponins occurring in the highest concentration. This is in line with the findings of Khoiroh *et al.*, (2022) who revealed the presence of alkaloids, flavonoids, saponins, steroids and tannins in *A. comosus* crown. Saponins are known for their antimicrobial, antifungal, and antioxidant properties, which may contribute to the preservation and quality enhancement of fish products when applied during processing.

Flavonoids were the second most abundant group of phytochemicals. Their presence is significant as flavonoids are well-documented antioxidants that scavenge free radicals and reduce lipid peroxidation (Akinmoladun *et al.*, 2010). This suggests that *A. comosus* crown extract could play an important role in reducing oxidative deterioration in smoked fish fillets, thereby prolonging shelf life and enhancing organoleptic quality. Tannins were also detected in appreciable amounts. These compounds have antimicrobial and astringent properties, which may aid in inhibiting bacterial growth and improving the texture of treated fish products (Trease and Evans, 2002). Alkaloids were present in moderate quantities (1.05%), and their antimicrobial and therapeutic effects have been widely reported, making



them important contributors to the bioactivity of the extract. Glycosides were recorded in the lowest concentration (0.22%) and may still play a role in contributing to the antioxidant potential.

The phytochemical profile of *A. comosus* crown indicates that it possesses antimicrobial and antioxidant properties, which supports its use as a natural preservative in fish processing. This aligns with the observations of Nwachukwu *et al.*, (2014), who demonstrated that pineapple extracts exhibited inhibitory effects against foodborne pathogens.

Bacterial Load of Raw *Clarias gariepinus* and *Malapterurus electricus* Fillets

The bacterial load of raw *Clarias gariepinus* and *Malapterurus electricus* fillets in Table 2 revealed no significant difference between the two species. *M. electricus* recorded a slightly higher count. The values obtained for *C. gariepinus* in this study (7.07×10^4) are lower than the values (1.4×10^6) reported by Oladipo (2013). The microbial levels obtained in this study fall within the acceptable limits for fresh fish as recommended by the International Commission on Microbiological Specifications for Foods (ICMSF, 1986), which sets the upper acceptable limit at 5×10^5 cfu/g for fresh fish.

The presence of bacterial load in both species is expected, as fresh fish is extremely perishable and is subject to bacterial spoilage due to poor handling (Getu *et al.*, 2015). The slightly higher bacterial count in *M. electricus* may be attributed to species-specific differences in skin mucous composition and handling, which can influence microbial adherence and growth, as reported by Okonko *et al.* (2008). The values from this result were within safe limits, the observed bacterial loads show the importance of proper preservation and processing. Without proper processing such as freezing, these initial microbial levels could multiply which can lead to spoilage and potential health hazards. This observation agrees with the findings of Olafsdóttir *et al.* (1997), who noted that the shelf life of fresh fish is directly related to the initial bacterial load and storage condition.

Effect of different Concentration of Salt and *Ananas comosus* Crown Solution on the Bacterial Load of Freshly Smoked *Clarias gariepinus* and *Malapterurus electricus* Fillets

The bacterial load of freshly smoked *Clarias gariepinus* and *Malapterurus electricus* fillets in Table 3 was influenced by both the concentration of salt and *Ananas comosus* solution, as well as by storage duration. The bacterial load of the smoked stored samples increases as the length of the storage period. The final value of bacterial load in this study falls within the maximum recommended range (5×10^5 cfu/g) of bacteria count for good quality fish products as recommended by Microbiological Guidelines for ready-to-eat food (i.e. $<10^6$ cfu/g) by Centre for Food Safety, Food and Environmental Hygiene Department (2007). The spoilage in the smoked fish sample is attributed to increased microbial load with storage duration since microbes play a

vital role in the spoilage of fish and fish products (Eyo, 2001). Some bacteria are commonly associated with fish under storage conditions (Abolagba and Igbinevo, 2010).

The combined data shows a consistent reduction in microbial counts with increasing concentrations of salt and *A. comosus* crown solution when compared to the untreated control. Treatments with 10–15% salt and *A. comosus* crown solution showed the lowest bacterial loads throughout the storage period. The values obtained in this study is similar to the values reported by Kwala *et al.*, (2024) in a Studies on microbial load of *Clarias gariepinus* smoked with different parts and product of *Parkia biglobosa*.

The antimicrobial activity of salt is primarily due to its dehydrating and osmotic effects, which reduce water activity and inhibit microbial enzyme systems. Similarly, the antibacterial properties of *A. comosus* crown solution may be attributed to its rich composition of phenolic compounds, flavonoids, and bromelain, which can disrupt bacterial cell membranes and inhibit metabolic activities (Vega *et al.*, 2024). These findings align with previous reports by Li *et al.*, (2012), who observed a significant decline in microbial counts in fish treated with natural plant extracts during storage. Studies have shown that plants 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 the spoilage of foods and food-borne illness (Bukar *et al.*, 2010).

Monthly variations revealed that bacterial load increased from February to June in both species, reflecting the combined influence of prolonged storage and elevated ambient temperature, which favor microbial proliferation. This is similar to the findings obtained by Dutta *et al.* (2018).

Samples treated with higher concentrations of salt and *A. comosus* crown solution shows a slower rate of microbial growth, indicating that these treatments extended the shelf life of the smoked fish. Similar seasonal increases in bacterial load have been reported by Kwala *et al.*, (2024) who linked them to the long storage period.

When comparing the two species, *M. electricus* fillets generally exhibited lower bacterial counts than *C. gariepinus*. This may be due to species-specific differences in proximate composition especially the lower moisture of *M. electricus* which limit microbial nutrient availability and growth potential.

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). During smoking period, smoking kilns used and overloading of the fishes on the smoking trays might lead to improper smoking and processing which can support bacterial and fungal growth. The environment in which smoked fishes are being displayed in the markets are not always hygienic, they are being

displayed on contaminated tables, near refuse dump, dirty gutter. The method of selling also contributes seriously in contamination of the smoked fish in which the smoked fishes are being touched with unwashed hands of the prospective customers. This encourages bacteria and fungi attack and subsequent production of toxins. This is in agreement with report of Bukola *et al.*, (2008)

Prevalence of Bacterial Isolates of Freshly Smoked *Clarias Gariepinus* and *Malapterurus electricus* Fillets Treated with Different Concentration of Salt and *Ananas comosus* Crown Solution

The results on the prevalence of bacterial isolates from freshly smoked *Clarias gariepinus* and *Malapterurus electricus* fillets in table 4 and 5 revealed variations in the types and occurrences of bacterial species across the different treatments. The predominant bacterial species identified in both fish samples were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella spp.*, *Escherichia coli*, and *Salmonella spp.*, with *S. aureus* showing the highest overall prevalence. This is in line with the recommendations of ICMSF (1998) who reported that bacteria present in fishes are normally associated with those found in their natural environment and influenced by the season and the harvesting conditions. Ayeloja *et al.* (2013) also reported similar microbes to those identified in the present study.

In *C. gariepinus* fillets, *S. aureus* accounted for 41.39% of the total bacterial isolates, followed by *S. epidermidis* (22.66%), *Pseudomonas aeruginosa* (15.79%), and *Klebsiella spp.* (13.02%), while *E. coli* (5.00%) and *Salmonella spp.* (2.14%) occurred less frequently. A similar pattern was observed in *M. electricus* fillets, where *S. aureus* (39.67%) also dominated, followed by *S. epidermidis* (19.68%) and *P. aeruginosa* (15.93%).

The lower prevalence of *E. coli* and *Salmonella spp.* suggests good sanitary conditions during smoking and storage, as these are fecal indicators of poor hygiene (Kwala *et al.*, 2024).

The control samples of both species exhibited the highest bacterial counts across all isolates, which indicates that the absence of salt or *A. comosus* treatment favored microbial proliferation. The progressive reduction in bacterial load with increasing salt concentration corroborates the antimicrobial properties of salt, which reduces water activity and inhibits microbial growth. Likewise, treatments involving *A. comosus* crown solution also showed marked reductions in bacterial occurrence, particularly at higher concentrations (10% and 15%). This agrees with the report of Lubaina *et al.* (2019), who attributed the antimicrobial effect of pineapple extract to the presence of bioactive compounds such as bromelain, phenolics, and flavonoids that disrupt microbial cell walls.

The persistence of *S. aureus* and *S. epidermidis* even after treatment may be due to their thermotolerant nature and ability to survive in low-moisture environments.

Meanwhile, the low incidence of *Salmonella spp.* across treatments indicates that the smoking temperature and duration were sufficient to destroy most enteric pathogens.

CONCLUSION

The study has shown that *Ananas comosus* crown contains important phytochemical compounds such as saponins, flavonoids, tannins, alkaloids, and glycosides at levels significant enough to pose antibacterial effect. The bacterial loads of raw *Clarias gariepinus* and *Malapterurus electricus* fillets were similar before processing. Treatments with salt and pineapple crown solution significantly reduced the bacterial load of smoked fish fillets compared with the untreated control. Higher concentrations of the treatments were more effective in inhibiting bacterial growth. The predominant bacterial isolates identified were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella spp.*, *Escherichia coli*, and *Salmonella spp.* The findings indicate that both the salt and pineapple crown solution can improve the bacterial quality of smoked fish fillets. Therefore, pineapple crown solution may serve as a natural preservative in fish processing as the microbial levels obtained in this study fall within the acceptable limits for fresh fish (5×10^5 cfu/g) as recommended by the International Commission on Microbiological Specifications for Foods (ICMSF, 1986).

RECOMMENDATIONS

Based on the outcomes of this study, the following recommendations are made:

1. Extension programs should be conducted to educate artisanal processors on the preparation and safe use of *A. comosus* crown as a natural preservative to promote improved nutritional and hygienic standards in local fish production.
2. Application of 10 – 15 % *A. comosus* solution is recommended in order to forestall spoilage.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

