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Research Article

Antimicrobial Effects of Fenugreek (Trigonella foecum-graecum) Extract on Smoked Dried African Catfish *Clarias gariepinus* (Burchell, 1822)

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Abstract:

This study evaluated the antimicrobial effects of fenugreek (Trigonella foecum-graecum) extract solution on smoke-dried African catfish (*Clarias gariepinus*) stored at ambient temperature (32.0±2°C) over the period of 21 days. The experimental treatments were the control, 0.5%, 1.0%, 1.5% (w/v) fenugreek extract solutions. A total of 70 fish of average mean weight of 30.98±1.32g were degutted, washed and soaked in the extract solutions for 30 minutes and later smoked-dried for 12 hours. The smoked-dried fish treated with 1.0% solution of extract had the highest moisture content of 12.33%, followed by 11.39% recorded in fish treated with 0.0% solution of the extract. Highest crude protein of 49.09% was obtained in the control while the least (37.76%) was recorded in the fish treated with 0.5% fenugreek extract. There was no increase in microbial loads in all the treated samples except in the control which increased from 5×103 to 18×103. There was no significant difference in the general acceptability of all the treated samples. This study revealed that fenugreek extract can be used as a preservative against microbial activities on smoked-dried *Clarias gariepinus* thereby reducing nutritional and economic loss.

Key words: fenugreek extract, microbial activities, African catfish, general acceptability.

Introduction

Fish is highly perishable because it provides favourable medium for the growth of microorganism after death [1]. The emergence of antibiotic-resistant bacteria has become a global problem and has led to a search for alternative sources of antimicrobial agents. Inadequate storage and preservation techniques are responsible for up to 40% of the total catch going to waste. Many of the challenges associated with fish spoilage are as a result of the lack of proper storage and preservation, leading to low protein intake, post-harvest losses, and lack of adequate technology and skills in many tropical countries [2]. Composition of the microflora on newly caught fish depends on the microbial contents of the water in which the fish live. Fish microflora includes bacterial species such as *Pseudomonas, Alcaligenes, Vibrio, Serratia and Micrococcus* [3]. Microbial growth and metabolism are the major causes of fish spoilage which produce amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable off-flavors [4]. For unpreserved fish, spoilage is a result of gram negative, fermentative bacteria (such as *Vibrionaceae*), whereas psychrotolerant gram-negative bacteria (such as *Pseudomonas spp. and Shewanella spp*.) tend to spoil chilled fish [3].

Fenugreek *(Trigonella foenum-graecum Linn*) is a legume belonging to the Fabaceae family. Nearly 175 compounds have been identified in fenugreek seeds [5]. Fenugreek contains active constituents such as steroid saponin compounds, fibers, phenolic acid compounds, protodioscin, flavonoids, hydrocarbons, alkaloids, terpenes, fatty acids glycosides, carbohydrates, amino acids, and their derivatives [6]. Fenugreek is rich with a wide variety of metabolites such as tannins, alkaloids, flavonoids, terpenoids and glycosides which are known to have antimicrobial properties [7]. Studying the antimicrobial effects of fenugreek extract on smoked-dried *Clarias gariepinus* can provide insight into its potential as natural preservative against microbial activities. Thus, this study aimed at evaluating the antimicrobial effects of fenugreek (*Trigonella foecum-graecum*) extract solution on smoke-dried African catfish (*Clarias gariepinus*).

Materials and Methods

Study Area

The study was conducted in fish processing unit of the Department of Fisheries, Faculty of Agriculture, University of Maiduguri, Borno State, Nigeria.

Experimental fish

A total number of 70 *Clarias gariepinus* were purchased from Custom fish market Maiduguri, Borno State and conveyed in a polythene bag to the laboratory for the experiment.

Procurement of the Fenugreek

Fenugreek (*Trigonella foecum-graecum*) were procured from Custom market, Maiduguri, and was carried in a polythene bag to the fish processing unit of the Department of Fisheries, University of Maiduguri, Nigeria.

Formation of extract

Different solutions were prepared by adding separately specific quantity (5g, 10g and 15g) of the fenugreek powder to make 0.5%, 1.0% and 1.5% concentration respectively, then discovered in 1000ml of distilled water and were allowed to stay for 24 hours to form 3 treatments [1]. No extract was added to the control.

Experimental design

The experimental fish were randomly assigned to one control and three experimental treatments. They were divided based on the concentration of the extract in the solution to 0%, 0.5%, 1% and 1.5%. Each treatment and the control were triplicated with total weight of 500g fish. The fish were soaked into the aqueous solution of the bitter leaf extract for 30 minutes. Thereafter, the fish were placed on wire mesh and allow to drain under shed. Light was set for the smoking kiln to glow for ten minutes. Then the fish were arranged based on their treatments and replications in the smoking kiln consisting of three racks and hard wood was used for ignition. The smoking process lasted for 12 hours and the fish were weight at the first 30min and after each 1 hour for all the smoking period until a minimum weight was obtained and the temperature was regulated between 45-85^{o}C [1]. After the smoking the smoked-dried fish were allowed to cool and packed in different containers based on the treatments of the extract and then transferred to a cool dry place, save from any contamination. Samples were taken for microbial analysis at seven days' interval for a period of 21 days.

Proximate composition determination

The proximate composition of the fish samples, before and after smoking were determined. The percentage of moisture content, crude protein, crude fibre, ether extract or lipid, ash, carbohydrate or nitrogen free extract (NFE) were determined as described by AOAC [8].

Moisture content

This was done based on the difference between the net weight and the weight after drying to a constant (at 100^{o}C) for 24 hours. Empty clean beakers, one for each sample were dried in a hot air oven at 100^{o}C for 30 minutes. Each beaker was then weighed (W1). Five grams of the sample were placed in the beaker and then reweighed (W2). The beaker with the sample was then dried in an oven at 60^{o}C for 72 hours, for a constant weight to be attained, and then transferred to a desiccator to cool. The beaker was quickly weighed with minimum exposure to the atmosphere (W3). The loss in weight of the sample during the drying represented the moisture content, while the dry matter was the weight obtained after drying the sample.

$$\% \text{ Moisture} = \frac{W2 - W3}{W2 - W1} \times 100$$

Crude protein

The crude protein content was analyzed using Kjeldahl tablets and 1gram of the samples was weighed into a digestion tube and Kjeldahl tablets were added. 10ml of concentrated Sulphuric acid (H_2SO_4) was added onto the tube and digested at 420°C, for 5 hours. After cooling, 80ml of distilled water was added into digested solution. About 50ml of 40% Caustic Soda (NaOH) was added to 50ml of the digest and then placed on the heating section of the distillation chamber. 30ml of 4% boric acid, bromocreasol green and methyl red (as an indicator) were put into a conical flask and placed underneath the distillation chamber for collection of ammonia, the solution changed from pink to green colour. About 0.1 normal solution of hydrochloric acid (HCL) was weighed into a burette. The conical flask containing the solution was titrated until the colour changed from green to pink. The burette reading was taken. The crude protein was calculated using the formula:

$$\% \text{ CP} = \frac{(A - B) \times N \times F \times 6.25 \times 100}{\text{Weight of sample}}$$

Where:
A = Volume of acid used for titrating the samples (in ml)
B = Volume of acid used for titrating blank sample (0) in ml

N = Normality of acid used for titration
F = Factor (14.007)
6.25 = Constant

Ether extract

Ether extract was determined by using Soxhlet apparatus, 1g of the sample was weighed into filter paper and 100ml of petroleum ether was measured and added into a flat bottom flask, this solution was heated at 45°C for 2hours. The collecting flask was removed, cooled in desiccator for 15 minutes and the percentage (%) fat in the sample determined using the formula:

$$\% \text{ Fat} = \frac{\text{Weight of flask + fat after extraction}}{\text{Weight of samples}} \times 100$$

Ash

Ash content was determined by weighing 1gram of the samples into crucible and dried at 105°C for 24hours, then cooled in the desiccator for 15 minutes and weighed. This placed in a muffle furnace for 2 – 3 hours at 600 to 650°C, then cooled in a desiccator for 15minutes and weighed.

$$\% \text{ Ash} = \frac{\text{Loss in weight}}{\text{Initial weight}} \times 100$$

Nitrogen free extract (NFE)

Percentage free extract was determined by difference as described by Abdullahi *et al.* [9]

: % NFE = 100 – (% CP + % CF + % EE + % Ash)

Where:

CP = crude protein, CF = crude fibre, EE = ether extract

Total bacteria count

Total bacterial count was carried out using tenth fold serial dilution with normal saline. 9ml each of the normal saline were dispensed in a sterilized test tube and 1gram of the sample was weighed and transferred into a sterile universal bottle and 9ml of normal saline was added and shook well. Then 1ml was taken using sterile tests to make 10 fold serial dilution, then 0.1ml was transferred from the 3rd tube to a sterile dried nutrient agar and spread, it was later incubated at 37^{o}C for 24 hours. The colony was counted using colony counter and result was recorded and presented as colony forming unit (cfu) [1].

Identification of microorganism

The isolate were identified by conventional methods starting with grams staining briefly, using a sterile wire loop a drop of distilled water was put on the center of grease-free slide and a portion of colony was picked and emulsified into drop of sample and allow to air dry before fixing to gram stain, crystal violet will then be applied for 3 minutes. It was then be replaced with a gram's iodine for one minutes, prior to rinsing with water and application of 95% alcohol until no colour appeared on the flow. Slides were then be rinsed with water and safranin was applied for 1-2 minutes. This was followed by rinsing and air drying and then observed microscopically under x 100 emersion oil objective [1]..

Sensory evaluation

The smoked-dried fish were subjected to 10 people panel from the Department of Fisheries. Where students and some technologist assessed the comparison of these products, the comparison was carried out in terms of organoleptic characteristics, such as colour, appearance, flavor, taste, and general accessibility. It was requested to assess each organoleptic features of the smoked-dried fish products in accordance to a 7-point hedonic scale (7 = excellent, 6 = very good,5 = good, 4= fair, 3 = poor, 2 = very poor 1 = extremely poor), as desperate by Abdullahi *et al.*[1].

Data analysis

Data obtained was subjected to one way analysis of variance (ANOVA) using XLSTAT,version 2022, followed by Duncan pairwise comparisons which was used to separate significantly different means at a confidence interval of 95%.

Results and Discussion

Fenugreek seed extract (%) concentration

Parameters	FSE 0.0	FSE 0.5	FSE 1.0	FSE 1.5
Moisture	11.39±0.03 ^{c}	8.53±0.04^{c}	12.33±0.2 ^{b}	11.07±0.2^{c}
Crude Protein	49.09±0.56 ^{a}	37.76±0.3 ^{c}	42.80±0.3 ^{a}	47.23±0.02^{d}
Either extract	7.00±1.33 ^{b}	8.00±1.33 ^{b}	9.00±2.55 ^{a}	9.00±1.65^{a}
Ash	12.00±3.42 ^{a}	10.00±0.85^{b}	11.00±2.55 ^{b}	11.00±1.67^{b}
Nitrogen free extract	25.95±0.00 ^{c}	34.95±0.00 ^{a}	25.95±0.00 ^{b}	27.60±0.95^{b}

Means with the same superscript across rows were not significantly different (P>0.05)
Keys**:** FSE = Fenugreek seed extract

Table 1: Proximate composition of experimental fish treated with different concentration of Fenugreek **seed extract** (g/100g)

Proximate composition of smoked-dried *Clarias gariepinus* treated with different concentration of (0.0, 0.5, 1.0, and 1.5%) of fenugreek seed extract is presented in Table 1. Highest value of moisture content 12.33% was recorded in fish treated with 1.0% concentration of extract followed by 11.39% in sample treated with 0.0% solution of the extract. The lowest value of moisture content (8.53%) was in sample treated with 0.5% of the extract. The control samples recorded the highest crude protein content of 49.09% followed by samples treated with 1.5% solution with a value of 47.23%, while least was recorded in fish treated with 0.5% of the extract with a value of 37.76%. There was significant difference (P≤0.05) in the moisture, crude protein and nitrogen free extract content among all the treatments and the control observed in this study.

The results of proximate composition indicated that, the crude protein formed the largest quantity of the dry matter among all treatments and the control, this was in line with the report of Ajani *et al*. [10] who reported that, crude protein formed the largest quantity of dry matter in all fish. Also there was significant difference among all the treatments and the control, the control sample recorded the highest crude protein content of 49.09% followed by samples treated with 1.5% solution with a value of 47.23%. The lowest value of crude protein content was recorded in fish sample smoke in 0.5% of the extract at 37.767%. Abdullahi *et al*. [1], stated that, protein and ash content do not vary as often as fat. There was significant difference in ash content for all the samples, the sample treated with 0.5% of the extract had the least. Olayemi *et al*. [11] stated that ash is a measure of the mineral content of fish.

Fenugreek extract Concentration level (%)	Initial weight (g)	Final weight (g)	Weight Loss(g)	Percentage Weight loss
0.0	500	165	335	67%
0.5	500	170	330	66%
1.0	500	180	320	64%
1.5	500	200	300	60%

Means with the same superscript across rows were not significantly different (P>0.05)

Table 2: Difference in weight lost after twelve hours of smoking *Clarias gariepinus* with a solution of fenugreek extract

Table 2 shows the weight loss of Clarias gariepinus during smoking period of twelve hours at temperature of 85°C for the first hour and remaining eleven hours maintained the temperature of heat at 45-85°C for smoking trail. The result of the weight loss of Clarias gariepinus treated with different solution of Trigonella foecum-graecum extract before and after smoking for 12 hours was presented in (Table 2). The percentage of average weight loss of 63.75% obtained in this study was similar to the percentage of average weight loss of 63.34% reported by Agbabiaka et al. [12] in their research work on the nutritional and storage quality of catfish (Clarias gariepinus) smoked with Anthonotha macrophylla.

Fenugreek Concentration %	Total Viable Count (cfu/g)
0.0	5×10^3
0.5	2×10^3
1.0	2×10^3
1.5	1×10^3

Means with the same superscript across rows were not significantly different (P>0.05)
Keys: CFU/G colony-forming units per gram

Table 3: Microbial population (cfu/g) in fish smoked with different concentration of fenugreek extract

Microbial population in fish smoked with the different concentration of fenugreek extract is shown in Table 3. The fish treated 0.0% has the highest microbial load of about 5×10^3. And 1.5 which shows that is least and is recorded as 1×10^3 while 0.5 and 1.0 shows the same cfu/g of 2×10^3.

Extract Concentration level (%)	**1 day**	**7 days**	**14 days**	**21 days**
0.0	5×10^3	15×10^3	16×10^3	18×10^3
0.5	2×10^3	2×10^3	2×10^3	2×10^3
1.0	2×10^3	2×10^3	2×10^3	2×10^3
1.5	1×10^3	1×10^3	1×10^3	1×10^3

Means with the same superscript across rows were not significantly different (P>0.05)

Table 4: Changes in Microbial loads (cfu/g) of smoked-dried *Clarias gariepinus* during storage at room temperature for twenty-one (21) days

Changes in microbial loads of smoked-dried *Clarias gariepinus* treated with different concentration of *Trigonella foecum-graecum* extract during storage at room temperature for the period of twenty-one (21) days is presented in Table 4. After one week (7) of storage, the population of bacterial loads decreased in all the treated samples except in control which increased from (5×10^3) to (18×10^3). Smoked-dried *Clarias gariepinus* treated with 1.5% solution of the bitter leaf extract was recorded the least microbial loads (1×10^3) after the storage period. In the changes in microbial loads of smoked fish products during storage at room temperature for the period of twenty-one (21) days, the result agreed with work of Abdullahi *et al*. [1]. After one week of storage, the population of microbial loads decrease in all the treated samples except in control which increase to 15×10^3 cfu.

Parameters	**0.0**	**0.5**	**1.0**	**1.5**
Appearance	5.00 ± 1.15^c	5.00±1.15c	6.00±1.15	7.00 ± 1.15^a
Colour	6.00 ± 0.09^b	6.00±0.09b	5.00 ± 0.09^b	7.00 ± 0.00^a
Flavour	$6.00\pm0'08^b$	5.00±0.08c	5.00 ± 0.08^c	7.00 ± 0.00^a
Texture	6.00 ± 0.43^a	6.00±0.43a	6.00 ± 0.43^a	6.00 ± 0.00^a
Aroma	5.00 ± 0.08^b	5.00±0.08c	5.00 ± 0.08^c	7.00 ± 0.00^a
General acceptance	7.33 ± 0.11^a	7.00 ± 0.11^a	7.00 ± 0.11^a	7.33 ± 0.00^a

Means with the same superscript across rows were not significantly different (P>0.05)

Table 5: Sensory evaluation of smoked Clarias gariepinus treated with different concentration Fenugreek Extract

The sensory evaluation scores as presented in Table 5 indicated that smoked catfish retained very good scores for appearance, color, flavor, texture, and general acceptance after 21 days of storage. The overall acceptability scores of the fish treated with 10g and 15g of fenugreek extract solution showed that the product was generally accepted. The results are in agreement with previous research [1] which found that deterioration in the eating quality of smoked fish can be best tested using organoleptic methods. The improved shelf life up to 21 days may be due to the active component of the fenugreek extract, which reduced water activity and impaired the action of spoilage microbes. This study revealed that incorporating fenugreek extract on fish before smoking has favorable effects on the overall quality of the final product, such as prevent microbial activity, enhanced taste, color, flavor, and general acceptability. These qualities are important for attracting consumer attention and improving the marketing strategy.

Conclusion

In conclusion, there was no increase in the microbial loads in all the treatments except in the control. Highest crude protein content (49.09±0.56.) was recorded in the control while the least value (37.76±0.03%) was obtained in the fish treated with 0.5% concentration of the extract. Sensory evaluation revealed that, the smoked-dried Clarias gariepinus retained good score of overall acceptability. There was significant influence of fenugreek extract on antimicrobial activity of smoked-dried Clarias gariepinus making it naturally suitable and microbial stable, thus limiting economic loss and possible health risk to consumers. Therefore, it is recommended to be used as natural preservative in fish processing and preservation industries.

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