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Effects of Indigenous spices on Microbial Quality and Sensory Attributes of Smoked Clarias gariepinus

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Abstract

This study investigated the influence of various indigenous spices on microbial load and sensory qualities of smoked African catfish (*Clarias gariepinus*). Twelve live specimens (1 kg) were processed and smoked using sterile protocols. Smoked samples preserved with individual spices were stored in sealed containers for two weeks. Microbiological analysis was performed biweekly on 1 g muscle tissue. Onion-spiced samples recorded the highest moisture content (39.23±0.46%), while garlic-spiced samples exhibited elevated total coliform counts ($77.6\times10^2\pm1.16$ cfu/g). *Salmonella*-related strains (including *Shigella*) were detected across treatments, alongside increased *E. coli* and *S. aureus* in week two. Organoleptic assessment revealed highest acceptability for mixed spice-treated fish (1.50±0.60). Indigenous spices improved sensory characteristics while exhibiting antimicrobial potential.

Keywords: African catfish, indigenous spices, microbial load, sensory evaluation, smoked fish

INTRODUCTION

Fish represents a significant source of food, income, employment and recreation globally. It is vital source of animal protein for both humans and livestock, particularly in developing nations (Ozigbo et al., 2014; FAO, 2014; Amponsah et al.,2016). However, its highly perishable nature due to microbial and enzymatic degradation limits its nutritional value (FAO,2016; Modibbo et al., 2014).

Preservation using natural additives with antimicrobial and antioxidant properties can enhance organoleptive quality and reduce harmful residues. Naturally occurring phytochemicals -such as polyphenols, flavonoids, tannins-found in spices like garlic (Allium sativum), ginger (Zingiber officinale), and lime (Citrus aurantifolia) have demonstrated preservative properties (Kumolu-Johnson & Ndimele, 2011). Garlic and ginger, in particular, exhibit a broad spectrum of bioactivities, including antimicrobial and antioxidant effects (Duvar et al.,2019; Cerviche, 2012).

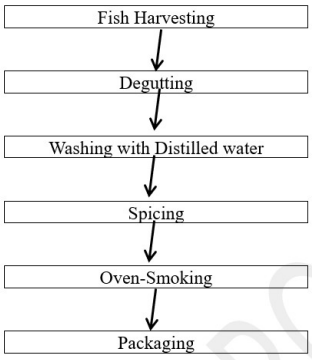
These locally available spices were employed to investigate their influence on the microbial and organoleptive characteristics of smoked Clarias gariepinus over a 14-day period.

MATERIALS AND METHODS

Experimental Procedure

This project was carried out in the Teaching and Research Farm of the Department of

Fisheries and Water Resources, University of Energy and Natural Resources Sunyani-Ghana. Twelve live specimens of Clarias gariepinus (mean weight: 1 kg) were procured for this study. Fish were slaughtered, de-gutted and washed under sterile conditions. Smoking was performed using teak wood (Tectona grandis). Muscle sample of 1g was collected from each hot spiced-smoked catfish twice at seven days interval 14 days at the temperature 90ºC. The microbial loads and proximate analyses of the spiced-smoked samples of the C. gariepinus were undertaken at the clinical and food science laboratories of Kwame Nkrumah University of Science and Technology (KNUST). A flow chart showing the processing method of the spiced-smoked *Clarias gariepinus* is seen in Figure 1.



Fish samples were washed thoroughly with dechlorinated and distilled water. They were thereafter spiced with ginger, garlic, onion, salt, mixed spices and one unspiced which served as the control treatment. One gram of each of the additives: ginger, garlic, onion, salt and mixed additives was used in spicing the previously weighed fish using Ahmed (2019). Teak *Tectona grandis* of 14 kg was used as the fuel wood for smoking experimental fish in Ahotor oven for about 90oC in 6 hours with a thermocouple (Thermo Sensors Corporation, Garland, Texas, United States of America).

Sampling and Transportation of spiced-smoked fish samples

The fish samples were collected under sterile conditions for two consecutive weeks. The samples were collected into sterile sample bags and labelled with codes for identification and transported to the Biotechnology Laboratory of the Department of Biochemistry, Kwame Nkrumah University of Science and Technology for analyses.

Chemical Reagents

The agars used were products of OXOID Laboratories, Basingstoke Hampshire, and England. They included Mannitol Salt Agar (MSA) for isolation of Staphylococcus, Brilliance E. coli selective agar for the isolation of E. coli, Xylose lysine deoxycholate agar for Salmonella isolation and MacConkey agar (MA) for total coliform count.

Serial Dilution

Serial dilution was done to reduce a dense culture of cells to a more usable concentration. A weighted mass of 5 g of each sample was weighed and placed in 45 mL of sterile peptone water solution. Serial dilution was performed to obtain a six-fold dilution (10-6) by transferring one milliliter (1 mL) of the stock into nine milliliters (9 mL) of sterile diluent and repeated for subsequent dilutions.

Quantitative Analysis

The quantitative assay was carried out to determine the microbial population of the samples as an index of quality and safety. The parameter considered was total Coliform count. Quantitative quality index is used for organisms that are regarded as mildly pathogenic thus would require microbial counts exceeding certain thresholds in a host to trigger physiological responses or symptoms.

Determination of Total Coliform Count (TCC)

Aliquots of one hundred microliters (100L) from each of the dilutions were inoculated into Petri dishes containing MacConkey agar. The inoculum was evenly spread with a sterile bent rod and allowed to dry for 15 minutes at room temperature. The plates

were inverted and incubated at 37ºC for 24 hours. The plates were observed for the presence of visible colonies, which were enumerated and kept for an additional 24 hours subsequent to a second enumeration to capture late maturing colonies. The plates were inoculated in triplicate as was done for the TAC.

Qualitative Analysis

The qualitative assay was carried out to isolate and identify specific organisms of interest, mainly food pathogens which are indicator of food quality and safety. The organisms considered in this study include *Staphylococcus aureus, Escherichia coli and Salmonella typhi*. These Gram-positive and negative food pathogens were selected as indicators of human-food contact contamination as well as indicators of hygiene and aseptic practices.

Determination of Staphylococcus aureus (Szermer et al., 2014)

Staphylococcus species were isolated and enumerated by the spread plate method and grown on Salt Mannitol Agar (SMA). One milliliter aliquot from each of the dilution were inoculated into already prepared Petri dishes of MSA. The inoculum was evenly spread with a sterile bent rod and allowed to dry for 15 minutes at room temperature. The plates were inverted and incubated at 35ºC for 24 hours. After incubation, yellow colonies were counted and recorded as *Staphylococcus* counts.

Determination of E. coli (Morcatti et al., 2015)

The presence of *E. coli* was determined by spreading an aliquot of 0.1ml of stock dilution of the samples on sterile plates of Brilliant *E. coli* selective agar and incubating at 37°C for 24 hours. The presence of *E. coli* was confirmed with the observation of purple colonies.

Determination of *Salmonella typhi* (Sohrabi et al.,2022)

The presence of Salmonella typhi was determined following a three-step procedure: pre-enrichment in 1% peptone water (5 g sample in 45 mL) at 37°C for 24 hours; selective enrichment in 9 mL Rappaport-Vassiliadis broth for 24 hours; and plating on Xylose Lysine Deoxycholate (XLD) and Brilliant Green Agar (BGA). After incubation at 37°C for 24 hours, presumptive Salmonella colonies appeared black on XLD (due to H2S production) and red on BGA. Further biochemical confirmation was recommended (Sohrabi et al., 2022).

Proximate Analyses

This was carried out using standard methods of Association of Analytical Chemists (AOAC, 1995) to determine the moisture content, ash content, crude protein, crude fat and carbohydrates contents of the samples of the spiced-smoked African catfish *Clarias gariepinus*.

Moisture Content

Five grams of each of the spiced-smoked samples was transferred to a previously dried and weighed dish. The dish was placed in an oven that was thermostatically controlled at 105°C for 5 hours. Afterwards, it was removed and placed in a desiccator to cool to room temperature and weighed. It was later dried for 30 minutes, cooled down to ensure constant weight.

Ash Content

Five grams of each spiced-smoked fish sample was weighed into a tarred crucible. Preashing occurred before ignition and the crucible was weighed before and after ignition. The crucible was placed in a cool muffle furnace. This was ignited for 2 hours at 600°. The muffle furnace was turned off to cool to about 250°C. Desiccator was closed to allow the cooling of crucibles before weighing.

Crude Fat

A round-bottom flask of 250 mL was weighed previously and dried (air oven at 100° C). Five grams each of dried spiced-smoked fish sample was weighed to 22 × 80 mm folded filter paper. A small cotton wool was placed in a folded filter paper for prevention of sample loss. Afterwards, about 150 mL of petroleum spirit B.P 40-60° C was added to the round-bottom flask and the apparatus was assembled. The condenser was connected to the Soxhlet extractor (manufactured by Infitek) and refluxed for 4 - 6 hours on the heating mantle. After extraction, the folded filter paper was removed and the solvent was recovered by distillation. The flask and fat/oil were heated in an oven at about 103°C to evaporate the solvent. The flask and contents were cooled to room temperature in a desiccator. Afterwards, the flask was weighed and the determined weight of fats/oils was collected.

Crude Carbohydrate

Two grams of each sample was weighed from crude fat determination into a 750 mL Erlenmeyer flask. 200 mL of 1.25% H2SO4 was added and the flask was immediately set on hot plate and connected to a condenser. The contents were boiled within one minute of contact with the solution. At the end of 30 minutes, the flask was removed and immediately filtered through linen cloth in funnel and washed with a large volume of water. Filtrate was washed (containing sample from acid hydrolysis) back into flask with 200 mL 1.25% NaOH solution. Flask condenser was connected and boiled for exactly 30 minutes. This was filtered through Fischer's crucible and washed thoroughly with water and 15 mL 96% alcohol was added. Crucible and contents were dried for 2 hours at 105°C. This was cooled in a desiccator and weighed. Crucible in a furnace was ignited for 30 minutes, cooled and reweighed.

Protein Determination

To the digestion flask, 2 g of sample and a half of selenium –based catalyst tablets and a few anti-bumping agents was added. Afterwards, 25 mL of concentrated H2SO4 was added and shaken until the sample was thoroughly wet. Flask was placed on digestion burner and heated slowly until boiling ceased and the resulting solution cleared. Thereafter, cooling was carried out to room temperature. Digested sample solution was transferred into a 100 mL volumetric flask and made up to the mark. To 250 mL conical flask, 25 mL of 2% boric acid was pipetted and 2 drops of mixed indicator (methyl red and methylene blue) was added.

The conical flask and its contents were placed under the condenser in such a position that the tip of the condenser was completely immersed in solution. 10 mL of the digested sample solution was measured into the decomposition flask of the Kejdahl unit, this was fixed and 40% NaOH (about 15-20 mL) was added to it. Ammonia produced was distilled into the collection flask with the condenser tip immersed in the receiving flask till a volume of about 150 mL–200 mL was collected. Before distilling another sample and on completion of all distillations, the apparatus was flushed as in step 1. Steam was passed only until 5 mL of distillate was obtained.

distillate was titrated with 0.1N HCL solution. The acid was added until the solution was colorless. Additional acid was added and the solution became pink. Nitrogen content was determined and duplicated and run a blank determination using the same amount of all reagents as used for the sample. The blank was used to correct traces of nitrogen in the reagents and included digestion as well as distillation.

Organoleptive Procedure

All the fish samples were stored at ambient temperature and collected for organoleptic assessment after twenty-four hours of smoking. The sensory quality attributes were evaluated based on a 5-point hedonic scale. The parameters examined were taste, flavor, texture, and appearance; and the following grades were allotted: $8 \leq 10 =$ excellent, $6 \leq 7 =$ very good, $4 \leq 5 =$ good, $2 \leq 3 =$

bad, and ≤ 2 = very bad.

A total of 20 questionnaires were distributed and administered among lecturers, students and non-teaching staffs, indicating organoleptic attributes of spiced-smoked African catfish (C. gariepinus). Valid information on age and gender was available for respondents.

Statistical Analyses

All data were analyzed using SPSS VERSION 16.0 and Minitab software to determine the difference in means and significant differences by using the analysis of variance and Tukey's test at ($P < 0.05$).

RESULTS

Organoleptic Attributes of Spiced-smoked African catfish *Clarias gariepinus*

From Table 1, the best taste (1.90±0.71) was observed in fish samples spiced-smoked with garlic. Onion spiced-smoked fish samples had the best flavor (1.90±0.91), while garlic- and ginger-spiced smoked fish samples recorded the best texture (1.80±1.00; 1.80±0.76), respectively, with significant differences ($P \leq 0.05$). For odor, the garlic spiced-smoked fish sample had the best outcome (1.80±0.76). The best appearing sample (1.60±0.75) was the ginger spice smoked treatment, the best overall acceptability was observed in the garlic and mixed spice smoked fish samples, with mean scores of (1.50±0.60) and (1.50±0.57), respectively, showing significant differences ($P \leq 0.05$).

Proximate Composition of differently Spiced-smoked African catfish (C. gariepinus)

Blood Serum Biochemistry

rom Table 2, the highest moisture content (39.23±0.46%) was recorded in onion spiced-smoked fish samples, while the .

Table 1: Organoleptic attributes of spiced-smoked African catfish (*C. gariepinus*

Treatment	Taste	Flavor	Texture	Odor	Appearance	Overall Accepta
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
GI	1.70 ± 0.80^{a}	1.70 ± 0.80^{a}	1.80 ± 0.76^{a}	1.65 ± 0.74^{b}	1.60 ± 0.75^{a}	1.35 ± 0.58^{c}
ON	1.50 ± 0.60^{b}	1.90 ± 0.91^{a}	1.55 ± 0.68^{b}	1.70 ± 0.73^{b}	1.50 ± 0.68^{ab}	1.40 ± 0.75^{b}
SA	1.40 ± 0.59^{c}	1.50 ± 0.76^{b}	1.55 ± 0.51^{b}	1.30 ± 0.47^{d}	1.45 ± 0.60^{b}	1.45 ± 0.51^{b}
GA	1.90 ± 0.71^{a}	1.85 ± 0.74^{a}	1.80 ± 1.00^{a}	1.80 ± 0.76^{a}	1.55 ± 0.75^{a}	1.50 ± 0.57^{a}
MI	1.70 ± 0.57^{a}	1.65 ± 0.58^{b}	1.50 ± 0.68^{b}	1.60 ± 0.68^{b}	1.35 ± 0.48^{c}	1.50 ± 0.60^{a}
CO	1.65 ± 0.87^{b}	1.50 ± 0.60^{b}	1.75 ± 0.63^{a}	1.40 ± 0.59^{c}	1.30 ± 0.47^{c}	1.30 ± 0.47^{c}

There were no significant differences ($P \leq 0.05$) among the means of all treatment groups.

Key:
GI = Spiced-smoked African catfish (*C. gariepinus*) with 1g of ginger (*Zingiber officinale*)
ON = Spiced-smoked African catfish (*C. gariepinus*) with 1g of onion (*Allium cepa*)
SA = Spiced-smoked African catfish (*C. gariepinus*) with 1g of common salt (Sodium chloride)
GA = Spiced-smoked African catfish (*C. gariepinus*) with 1g of garlic (*Allium sativum*)
MI = Spiced-smoked African catfish (*C. gariepinus*) with 0.25g each of GI, GA, ON & SA
CO = Control (Unspiced smoked African catfish, *C. gariepinus*)

lowest (20.13±0.08%) was observed in the control, with significant differences (P≤0.05). Mixed and salt spiced-smoked samples had the highest crude fat values (19.36±0.71%; 19.96±0.41%), respectively. The total ash content was highest (8.46±0.52%; 7.37±0.28%) in samples spiced-smoked with salt and ginger, while the lowest (4.22±0.08%) was recorded in garlic spiced-smoked fish samples.

However, the highest crude protein values were observed in the control (58.10±0.80%) and mixed spiced-smoked fish samples (58.57±0.09%), compared with other treatments, with significant differences (P≤0.05). There were also significant differences (P≤0.05) in the total carbohydrate contents across all spiced-smoked fish samples, with garlic spiced-smoked fish having the highest (4.92±0.91%) and the control the lowest (0.68±0.22%).

Quantitative Assessment of Fish Quality and Safety

Total coliform count (cfu/g)

The quantitative indicator considered was the total coliform count. The results obtained for the assays were compared to the safety standards and limits as determined by the Ghana Standards Authority (GSA) and the Food and Drugs Authority of Ghana (FDA) to establish the wholesomeness and safety of the fish samples for consumption.

Qualitative Assessment of Fish Quality and Safety

The organisms considered in the qualitative assessment of the quality and safety of the fish included *Escherichia coli* (E. coli), *Staphylococcus aureus* (S. aureus), and *Salmonella* spp. These foodborne pathogens are known to be implicated in spoilage and are also associated with food-related health conditions of moderate to severe symptoms.

Escherichia coli (E. coli)

From Table 4, the results obtained for the *E. coli* assay showed that there was an almost absolute absence in week 1, except in the case of smoked fish spiced with onion (ON), where some *E. coli* cells were detected with less than 100 cfu/g. On the contrary, an amplified *E. coli* population was detected in the samples during week 2.

Staphylococcus aureus (S. aureus)

The results in Table 5 revealed that the *S. aureus* assay was similar to that of *E. coli* in Table 4, with relatively higher populations of *S. aureus* detected in the samples analyzed in the second week. The data showed that samples analyzed had lower counts of *S. aureus* in the first week.

Table 2: Proximate composition of differently spiced-smoked African catfish (*C. gariepinus*).

Parameters	GA	GI	ON	SA	CO	MI
Moisture (%)	31.90 ± 0.16^{b}	24.78 ± 0.06^{c}	39.23 ± 0.46^{a}	21.22 ± 0.22^{d}	20.13 ± 0.08^{e}	14.51 ± 0.20
Crude fat (wet basis, %)	13.49 ± 0.00^{d}	18.22 ± 0.10^{b}	9.24 ± 0.30^{e}	19.96 ± 0.42^{a}	15.36 ± 0.18^{c}	19.36 ± 0.72
Total ash (%)	4.22 ± 0.08^{e}	7.37 ± 0.28^{ab}	5.17 ± 0.37^{de}	8.46 ± 0.52^{a}	5.74 ± 0.18^{cd}	6.69 ± 0.26^{b}
Crude protein (%)	45.47 ± 0.83^{b}	45.99 ± 0.92^{b}	44.58 ± 0.038^{b}	46.13 ± 0.13^{b}	58.10 ± 0.80^{a}	58.57 ± 0.09
Total carbohydrates (%)	4.92 ± 0.91^{a}	3.64 ± 1.22^{b}	1.78 ± 0.57^{b}	4.23 ± 1.95^{a}	0.68 ± 0.22^{c}	0.88 ± 0.62^{b}

Means that do not share the same letter are significantly different (P≤0.05).

Key: GA = Garlic (*Allium sativum*); GI = Ginger (*Zingiber officinale*); ON = Onion (*Allium cepa*); SA = Common salt (NaCl); CO = Control (Unspiced); MI = Mixed spices (0.25 g each of GI, GA, ON, SA).

Salmonella species (cfu/g) from differently spiced-smoked African catfish *C. gariepinus*

From the result revealed in Table 6, Salmonella spp. were not detected in any treatment group at both time points, indicating compliance with safety standards. Confirmation was validated using both the Oxoid Salmonella Latex Test and the tests outlined in ISO 6579:2017.

Table 3: Phytochemical analysis of Tephrosia bracteolata

Sample	Week 1	Week 2
GA	$77.67\times10^{2}\pm1.16^{a}$	$83.33\times10^{5}\pm0.58^{a}$
GI	$27.67\times10^{2}\pm0.58^{c}$	$9.53\times10^{5}\pm0.58^{c}$
ON	$7.63\times10^{2}\pm0.06^{e}$	$4.77\times10^{5}\pm0.06^{f}$
SA	$6.33\times10^{2}\pm0.06^{e}$	$6.37\times10^{5}\pm0.06^{e}$
CO	$9.47\times10^{2}\pm0.06^{d}$	$8.23\times10^{5}\pm0.12^{d}$
MI	$56.67\times10^{2}\pm0.58^{b}$	$37.33\times10^{5}\pm0.57^{b}$

Means that do not share the same letter are significantly different (P≤0.05).

Key: TCC = Total coliform count (cfu/g); GA = Garlic (*Allium sativum*); GI = Ginger (*Zingiber officinale*); ON = Onion (*Allium cepa*); SA = Common salt (NaCl); CO = Control (Unspiced); MI = Mixed spices (0.25 g each of GI, GA, ON, SA).

Table 4: *Escherichia coli* (cfu/g) from differently spiced-smoked African catfish (*C. gariepinus*)

Sample	Week 1 (cfu/g)	Week 2 (cfu/g)
MI	–	++++
SA	–	++++
GA	–	++++
GI	–	++++
CO	–	++++
ON	+	++++

Legend:
– = Negative or not detected
+ = Detected in units of 50 cfu/g
+ (¡ 100 cfu/g), ++ (¿50 $<$ 1000 cfu/g), +++ (¿1000 $<$ 10000 cfu/g), ++++ (¿10000 $<$ 100000 cfu/g)

Key:
E. coli = *Escherichia coli* (cfu/g)
GA = Spiced-smoked African catfish (*C. gariepinus*) with 1g of garlic (*Allium sativum*)
GI = Spiced-smoked African catfish (*C. gariepinus*) with 1g of ginger (*Zingiber officinale*)
ON = Spiced-smoked African catfish (*C. gariepinus*) with 1g of onion (*Allium cepa*)
SA = Spiced-smoked African catfish (*C. gariepinus*) with 1g of common salt (Sodium chloride)
CO = Control (Unspiced smoked African catfish, *C. gariepinus*)
MI = Spiced-smoked African catfish (*C. gariepinus*) with 0.25g each of GI, GA, ON & SA

Table 5: Staphylococcus *aureus* (cfu/g) from differently spiced-smoked African catfish (*C. gariepinus*)

Sample	Week 1 (cfu/g)	Week 2 (cfu/g)
MI	++	++++
SA	++	++++
GA	–	++++
GI	–	++++
CO	–	++++
ON	+	++++

Legend:
– = Negative or not detected
+ = Detected in units of 50 cfu/g
+ (¡ 100 cfu/g), ++ (¿50 < 1000 cfu/g), +++ (¿1000 < 10000 cfu/g), ++++ (¿10000 < 100000 cfu/g)

Key:
S. *aureus* = Staphylococcus *aureus* (cfu/g)
GA = Spiced-smoked African catfish (*C. gariepinus*) with 1g of garlic (*Allium sativum*)
GI = Spiced-smoked African catfish (*C. gariepinus*) with 1g of ginger (*Zingiber officinale*)
ON = Spiced-smoked African catfish (*C. gariepinus*) with 1g of onion (*Allium cepa*)
SA = Spiced-smoked African catfish (*C. gariepinus*) with 1g of common salt (Sodium chloride)
CO = Control (Unspiced smoked African catfish, *C. gariepinus*)
MI = Spiced-smoked African catfish (*C. gariepinus*) with 0.25g each of GI, GA, ON & SA

Table 6: *Salmonella* species (cfu/g) from differently spiced-smoked African catfish (*C. gariepinus*)

Sample	Week 1 (cfu/g)	Week 2 (cfu/g)
GA	–	–
GI	–	–
ON	–	–
SA	–	–
CO	–	–
MI	–	–

Legend: – = Negative or not detected

Key:
S. sp = *Salmonella* species (cfu/g)
GA = Spiced-smoked African catfish (*C. gariepinus*) with 1g of garlic (*Allium sativum*)
GI = Spiced-smoked African catfish (*C. gariepinus*) with 1g of ginger (*Zingiber officinale*)
ON = Spiced-smoked African catfish (*C. gariepinus*) with 1g of onion (*Allium cepa*)
SA = Spiced-smoked African catfish (*C. gariepinus*) with 1g of common salt (Sodium chloride)
CO = Control (Unspiced smoked African catfish, *C. gariepinus*)
MI = Spiced-smoked African catfish (*C. gariepinus*) with 0.25g each of GI, GA, ON & SA

Analyses of Organoleptive observation across all spiced-smoked fish samples

The result on figure 2-7 in terms of texture, also indicated that, smoked catfish (Clarias gariepinus) spiced with mixed (all treatments) was rated to have the highest percentage (60%) with an excellent appearance, followed by onion (55%), garlic (50%) salt (45%) and (40%) for ginger, whiles, control-unspiced smoked catfish (Clarias gariepinus) had the lowest percentage (35%) but with a very good appearance.

In terms of odor, smoked catfish (Clarias gariepinus) spiced with salt was rated as the best or having the highest percentage (70%) with an excellent appearance, followed by control (unspiced), (65%) ginger (50%), mixed (all treatment) (50%), onion (45%) whiles, smoked catfish (Clarias gariepinus) spiced with garlic had the lowest percentage (40%) but with a very good appearance.

The overall performance in terms of appearance from figure 2-7 indicated that, unspiced smoked catfish (Clarias gariepinus) known as the control had an excellent value (70%), while the overall treatments in terms of acceptability-taste, flavor, texture, and odor, salt recorded (88.2%) and it was indicated as the highest, followed by onion (70.2%) and control-unspiced (70.2%) as the second (2nd) highest, ginger recorded (68.4%), mixed-all treatment (66%) whiles garlic had the least (66.1%). The result shows slight difference in the appearance of figure 2, 4, 5 (onion, salt garlic as 60%) and 6 (mixed 65%) but figure 2 ginger (55%) differs greatly from figure 8 (70%).

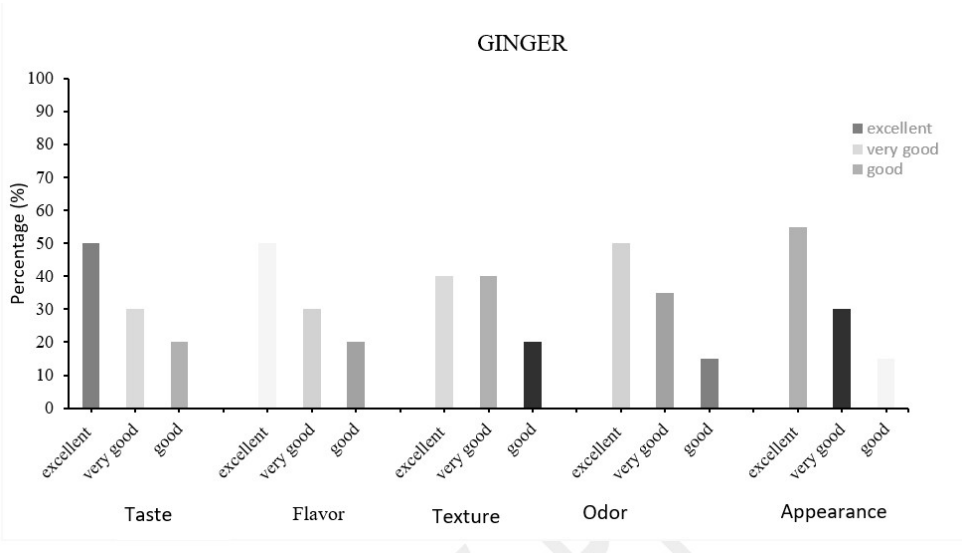


Figure 2: Effect of ginger as a preservative on smoked *C. gariepinus* stored at ambient temperature

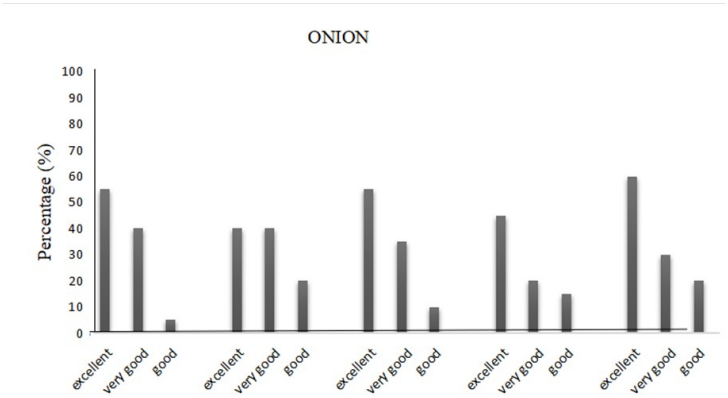


Figure 3: Effect of onion as a preservative on smoked *C. gariepinus* stored at ambient temperature

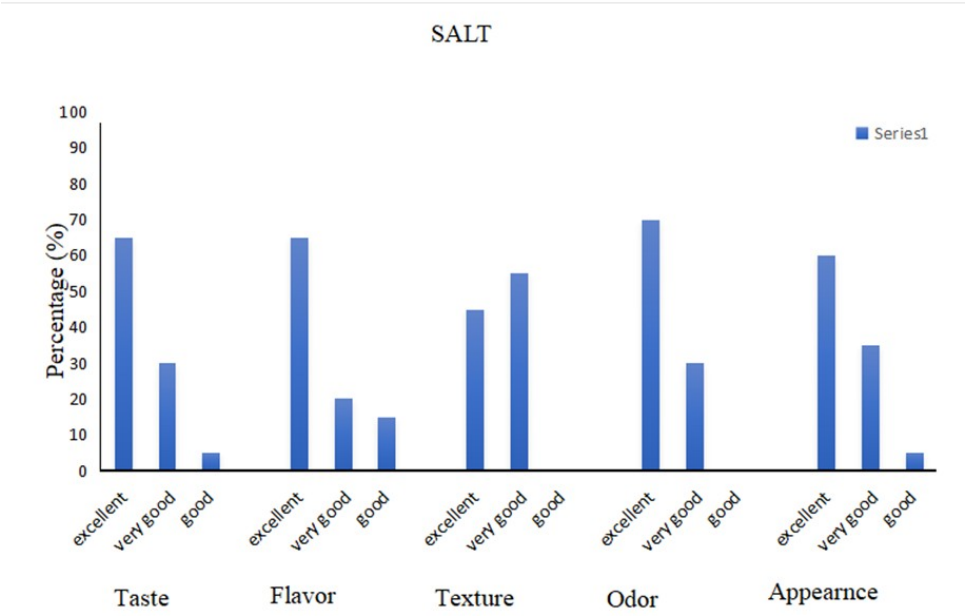


Figure 4: Effect of salt as a preservative on smoked *C. gariepinus* stored at ambient temperature

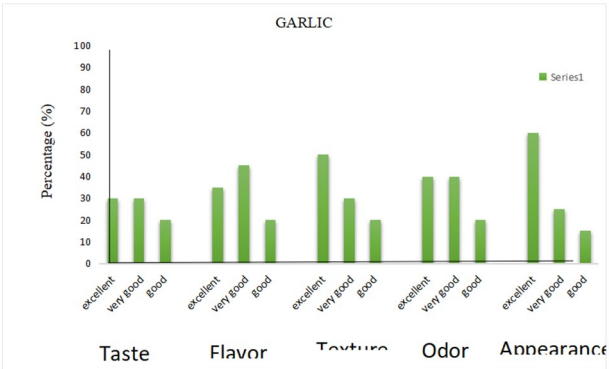


Figure 5: Effect of garlic as a preservative on smoked catfish stored at ambient temperature

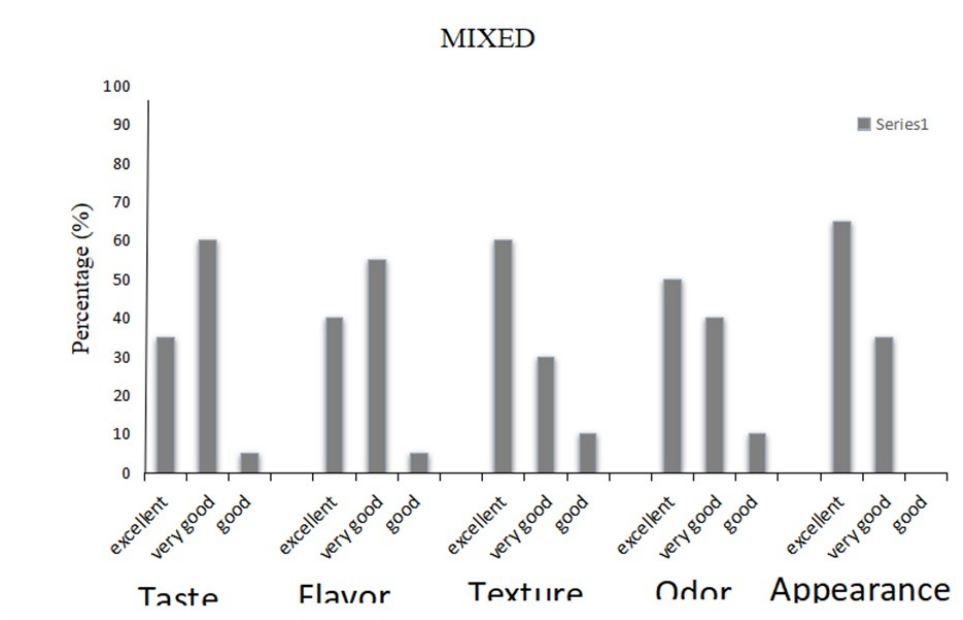


Figure 6: Effect of mixed (all treatments) as a preservative in smoked *C. gariepinus* stored at ambient temperature

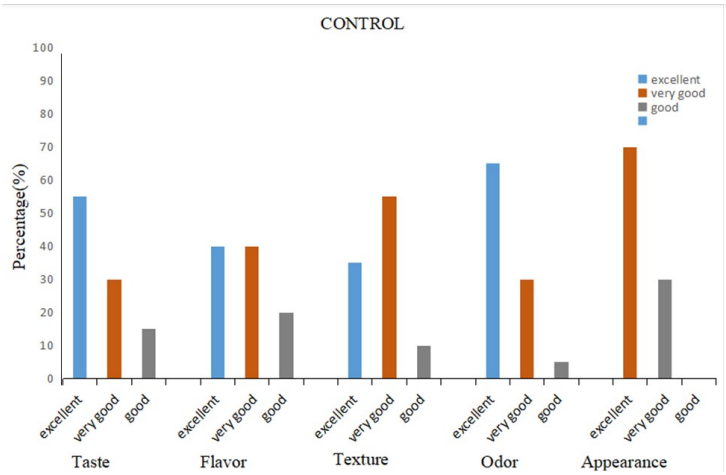


Figure 7: Organoleptic evaluation of unspiced (control) smoked *C. gariepinus* stored at ambient temperature

DISCUSSIONS

Microbial evaluation and proximate analyses of smoked fish samples

The study reports on the microbial counts of smoked African catfish (*C. gariepinus*) spiced with ginger, garlic, onion, salt, mixed spices, and control. The total coliform count (TCC) (cfu/g) heightened as the days increased. This agrees with the result of Dutta et al. (2018), who observed that microbial load increased in smoked fish (*Oreochromis mossambicus* and *Pangasius hypophthalmus*) stored over three weeks. This increased TCC may have been due to improper processing, inappropriate handling after post-processing, and the presence of moisture due to inadequate heat (Obodai et al., 2011). The findings of (Ayuba et al., 2013) corroborate these observations, reporting that the total bacterial load of smoked sardine (*Sardina pilchardus*) sold in some parts of Nigeria had high levels of bacterial load (0.988×10^3 to 2.632×10^3 cfu/g).

The counts obtained in the present study were above the safe limits of (1.0×10^2 cfu/g and 1.0×10^3 cfu/g for tolerable and maximum thresholds, respectively) as dictated by the Food and Drug Authority (FDA) and Ghana Standards Authority (GSA). The highest TCC (83.33×10^5 cfu/g) was obtained in week 2 with fish spiced with garlic. According to (FAO, 1979), good quality smoked fish should have a total bacterial count of less than 10^5 cfu/g and total coliform counts should not exceed 10 cfu/g to 100 cfu/g.

Staphylococcus aureus and *E. coli* both increased with storage time over two weeks. This agrees with the report of Okonta and Ekelemu, (2005), who confirmed the proliferation of *S. aureus* in smoked fish in some Nigerian markets. Furthermore, Kester et al. (2013), reported the increasing load of *S. aureus* in four batches of cold-smoked *Gadus morhua* at various storage intervals. The microbial loads of cold smoked Gadmus morhua increased as the moisture content increased. It was evidenced in this present study, as high moisture content was present in all the samples. Hence, its presence contributed to the growth S. aureus and E. coli. This result conforms to that of Eyo (2001) who reiterated that cold smoking of fish retains moisture in fish, this invariably reduces the shelf-life of smoked fish thereby proliferating the growth of micro-organisms.

The absence of Salmonella species indicated that the smoked fish was wholesome for human consumption. This was in contrast with the report of Yakubu and Ngueku (2015) who explained that there was presence of Aspergillus flavus and Aspergillus famigatus in smoked fish samples. Microorganisms like Salmonella species are extremely poisonous to man.

The smoking temperature 190 o C employed in this present study with the use of Ahotor oven enhanced the growth of E. coli, TCC and S. aureus during the 2 weeks storage. This was in contrast with the result of Hwang et al. (2019) who explained that before microbial contamination can be curbed in smoked fish the applied heat must exceed 600oC in temperate regions in order to destroy any inherent spores. Probably, the significant presence of moisture in smoked fish contributed to the increase in microbial loads over the 2 weeks of storage. Hence, the presence of E. coli and S. aureus may elicit diarrhea and urinary tract infections in man (Adelaja et al. 2013). There was no significant difference ($P_i0.05$) in the crude protein content of all fish samples which indicated that their nutritional profile was not negatively affected by the smoking temperature employed.

Organoleptive assessment of smoked fish samples The assessors' responses in this study revealed that the appearance of the spiced smoked fish was very crucial in their evaluation, alongside other sensory indicators such as texture, taste, flavor and odor. Indigenous spices add quality to smoked fish as seen in the result of this study. The salted smoked fish had the best acceptability due to its appearance, taste and odor, while the mixed spice smoked C. gariepinus exhibited the highest texture rating among all spiced smoked fish. These results were in tandem with those of Taniya and Kannan (2016), Iheagwara (2013) who reported that indigenous spices added to the organoleptic properties of smoked C. gariepinus in relation to taste, flavor, appearance and total acceptability. Ahmed et al. (2011), observed that the spiced fish with salt alone might not be sufficient to enhance the organoleptic properties of smoked C. gariepinus. This is in confirmation with the result of this study, as the mixed spiced-smoked C. gariepinus had the best texture among the spiced fish.

Certain spices exhibit antimicrobial and antioxidant properties, which may contribute to food safety and shelf stability hence, the absence of

Salmonella species in the smoked C. gariepinus in this study indicated that the spices had no harmful properties for human consumption. This was in consonance with the findings of Onyeagba et al. (2004) who observed that the addition of indigenous spices prolongs shelf-life and enhances organoleptic properties of smoked C. gariepinus.

The count obtained in the samples were above the safe limits of (1.0x102 cfu/g and 1.0x103) cfu/g for tolerable and maximum thresholds respectively as dictated by Food and Drug Board Authority (FDA) and Ghana standard authority (GSA). The highest (83.33x105) cfu/g TCC was obtained in week 2 with fish spiced with garlic. This study revealed that the appearance of the spiced smoked fish was very crucial in their evaluation, so also were other indices such as texture, taste, flavor and odor. Indigenous spices add quality to smoked fish as seen in the result of this study. The salted smoked fish had the best acceptability due to its appearance, taste and odor, while the mixed spice smoked C. gariepinus had the best texture quality among all spiced smoked fish.

It can be concluded that indigenous spices have preservative and organoleptic properties that can add value to smoked C. gariepinus. The addition of indigenous spices helps in enhancing the organoleptic properties of smoked C. gariepinus. Moreover, cold smoking increases microbial loads in smoked C. gariepinus.

RECOMMENDATION

Fish processors should be encouraged to use more of indigenous spices for fish processing due to their affordability and easy access to enhance fish organoleptic properties. Hot smoking should be encouraged in smoking C. gariepinus in order to reduce microbial proliferation during fish storage. Molecular confirmation of microbial species, spice concentration test and exploration of antioxidant levels should be carried out in future studies.

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