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Microbial Examination of Some Selected Natural Preservatives on The Shelf Life And Safety of Smoked Tilapia Fish (*Oreochromis niloticus*)

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Research Article

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ABSTRACT

Fish has been playing an important role in nutritional, profitability and livelihood security of people in many developing countries. This work was carried out to study the microbial examination of some selected natural preservatives on the shelf life and safety of smoked tilapia fish (*Oreochromis niloticus*). The microbial load counts (TVC, TCC and TFC cfu/g) of the microorganisms, sensory evaluation, proximate composition and isolated microorganism were evaluated on smoked tilapia fish preserved for 0, 2, 4, 6 and 8 weeks with 5 g of salt, ginger, garlic, clove (natural preservatives) and unpreserved sample. Salt sample recorded highest total viable count (TVC) of 3.5×10^5 and zero/no growth was recorded in ginger, clove and garlic sample at the end of 8 weeks of storage while after 6 weeks of storage, unpreserved sample got spoilt. Highest fungi count (TFC) of 4.4×10^5 was recorded in clove sample and zero/no growth was recorded in salt, ginger and garlic sample while unpreserved sample has spoilt after 4 weeks of storage. At the end of 8 weeks of storage, the total coliform count (TCC) of all the samples recorded zero/no growth except unpreserved sample that has been spoilt after 6 weeks of storage and the microorganism isolated from the samples exhibited that the highest number of microorganism species was showed in untreated sample with 6 species of microbes and the least was in garlic with 3 species of microbes. The highest value (64.75%) of crude protein in proximate composition was recorded in ginger sample while the least was 20.32% in unpreserved sample. Sensory evaluation results show that all the samples were generally accepted by consumers except unpreserved sample. A significant difference in the number of colonies formed between the groups at ($p<0.05$) was recorded in Statistical analysis. All the preservative samples used in this work, reduced the microbial load, discourage quick spoilage, and encourage longer shelf-life of smoked tilapia fish. These natural preservatives are medicinal which have no effect and add to the flavour/seasoning of some foods and they also act as antimicrobial agents by killing microbes present in the food.

INTRODUCTION

Fish is a highly nutritious food with high protein and is of high quality compared to those of meat and egg [1]. In Nigeria at present fish constitutes 40% of animal protein intake [2]. Fish is advantageous over pork or beef because fish are a cheap source

of animal protein with little or no religious rejection of it. The report by Glomset [3] stated that fish is currently being used as a good tool for food therapy and source of therapeutic substances for the treatment of coronary diseases, auto-immune diseases, protein energy malnutrition and anemia. To prolong the shelf life of fish, it is preserved by many preservation processes which including sun drying, solar drying, canning and application of heat (smoking) among others for the desirable consumption of fish. Dried fish is a major component of harvested fisheries by some farmers because of simplicity and the cost of the method in many countries including Nigeria. It has been reported that about 25 to 30% of the world fish catch is consumed in the dried, salted, smoked form or combination of these processes [4]. The exact shelf-life extension can be achieved by various preservation methods, such as, refrigeration, freezing, canning, salting, brining (wet salting), icing, smoking, glazing, drying, frying etc. with a suitable temperature.

At low temperature, refrigeration and freezing help in preserving fish from micro- organisms in which the fish remain free from spoilage for longer duration. Although some preservation methods such as canning and freezing are relatively expensive in developing countries while smoking is recommended as the simplest method that does not require sophisticated equipment or highly skilled workers [5]. For longer preservation of fish by smoking method is commonly acceptable by farmers because it reduces microbial stability, imparts desirable color and flavor. Due to water reduction activity in smoked fish, the shelf-life of smoked fish product is usually extended primarily. The dry fish could be safe from molds and bacteria infestation when the moisture content is less than 30% to ensure short shelf life of the fish product [6].

Baird-Parker, [7] reported that the microbial spoilage and chemical deterioration are responsible for loss of 25% of gross primary agricultural and fishery products every year. So therefore, spoilage of food products can be due to chemical characteristics, enzymatic activities or microbial activities. Water activity in the fish through smoking preservation method is lowered to the point level where the activity of spoilage microorganisms is inhibited [8]. However, the application of heat or excess of heat to dehydrate fish does not only remove water but also affect the nutritional content of the dried fish. Studies have shown that one of the effects of smoking is decrease in available lysine and this effect on lysine is proportional to the temperature and duration of smoking [6]. Although the cause of improper smoking and drying of fishes may lead to insect infestation, fragmentation, fungal attack and degradation of the product [6,9]. In Nigeria, smoked fish is relished or fondness food item in many dishes and to prevent any health hazard that may arise from consumption of unhealthy smoked fish so therefore it is important to monitor the microbiological quality of smoked fish. Among all the preservation methods only smoking is the preferred and acceptable method of fish preservation in most rural areas and riverine fishing communities because it is cheap and require less workers. Akinola et al. [10] described several methods of fish preservation in his research work and also reported the superiority of fish preservation by smoking in most fishing communities in Nigeria owing to non-availability of electricity. In another way, salting, brining, addition of vinegar and the type of wood used for the smoking fire have contributed to the quality of smoked fish and smoking combined with salting or brining for pickling reduced incipient spoilage resulting in extension of shelf life from two to six months [11-13].

In Nigeria, there is an increase in the consumption of smoked fishes among the people. And some people testified that they do not eat foods that are preserved unnaturally due to health risk. Some chemical preservatives like table salt, brining, addition of sodium sorbate are commonly used for smoking of fish in which they may have adverse effect on human health but herbal preservatives have no effect and add to the flavor and seasoning for some foods and they also act as antimicrobial agents by killing microbes that present in the food. According to the Wikipedia, The free encyclopedia stated that any naturally occurring or synthetic substance that is added to products such as foods, pharmaceuticals, paints, agricultural products, biological samples and wood to prevent decomposition by microbial growth or by undesirable chemical changes is known as preservative. Herbal preservatives also called natural preservatives are used in preserving food naturally. Some of these herbal preservatives consist of proteins, eugenol, gallic-acid, folate, tannis, vitamin B, vtamin C, vitamin E, vitamin K, choline and triterpene. Preservatives help to keep foods fresh, tasting longer, remain desirable color and prevent them from deteriorating and rotting too quickly. Synthetic or artificial preservatives help in processed and packaged of many foods sold in grocery, food industry and many stores for longer shelf-life. However, it is clear that many natural preservatives work just as well as synthetic ones. All natural preservatives have similar or different characteristics and uses that help in preserving and increase shelf-life of many foods. These include preservative like; Rosemary, Sage, Ginger, Cumin seed, Coriander seeds, Cinnamon, Cloves, Garlic, Cayenne pepper, Fennel seeds, Neem oil, Caraway seeds, Salt, Lemon juice, Pickling, Sugar and so on [14-18].

It is necessary to produce good quality and safe smoked seafood products in order to satisfy the consumer demand for the product. The result of microbial spoilage really affects the fish and fisheries products which are among the most perishable commodities worldwide. According to lund, [19] who stated that microbial activity is responsible for the spoilage of most fresh and of several preserved seafood's. So therefore, it is presumed that about one-third of the world's food production is lost annually as a result of microbial spoilage. Gram and Huss [20] also stated that the microbial contents of the water in which the fish live will determine the composition of the micro flora on newly caught fish. Nevertheless, the microbial growth and metabolism is a major cause of fish spoilage which produce some substances like amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with undesirable and unacceptable off flavors [21-23].

Gram-negative, fermentative bacteria such as (Vibrionaceae), psychrotolerant Gram-negative bacteria such as (*Pseudomonas* spp. and *Shewanella* spp.) result to spoilage of unpreserved fish and chilled fish [19,24] reported that gram-positive bacteria, Potential pathogens, coagulase-positive Staphylococcus, and Escherichia coli were detected in smoked fish samples from 4 local

Markets in Kainji Lake area of Nigeria. Therefore the purpose of this research work is to examine the effect of some selected natural preservatives on the microbial activity, physical appearance, nutritional quality, shelf life and safety of smoked tilapia fish during the period of 8-weeks of storage.

MATERIALS AND METHODS

Area of Study

The research work was carried out in Ibarapa central local government area, which has its headquarters in the town of Igboora covers about 440 km^2 of savannah zone to the west of Ibadan, the Ogun and Oyan rivers from its eastern and western boundaries respectively. It is between approximately latitude 7.530 and longitude 3.080 with the population of 102,979 according to 2006 census. The location of Oyo State College of Agriculture and Technology Igboora is within 70.5 North and 30.30 East of the equator with an average annual rainfall of 1278 mm and average annual temperature 27°c [25].

Collection of Fish and Preservative Samples

About Thirty kilogramme (30 kg) of fresh tilapia fish (*Oreochromis niloticus*) were purchased from Oyan dam in Ogun State of Nigeria. The tilapia samples (fish) were taken to the Department of Science Laboratory Technology of the Oyo State College of Agriculture and Technology, Igboora where the research work was carried out. Four natural preservatives such as salt, cloves, ginger and garlic were bought at a Towobowo local market in Igboora, Oyo State.

Processing of Natural Preservatives

The four natural preservatives (salt, cloves, garlic and ginger) used were rinsed thoroughly with tap water and air dried at a room temperature for 24 h. They were milled to fine powder with the aid of a Binatone blender (Model BLG+10), sieved and weighed, then poured inside an air-tight container until ready for use.

Grouping of the Samples (Smoked Tilapia Fish)

The samples (fish) were chosen randomly and divided into 5 portions. The samples were subjected to treatments. The treatments were as follows; Portion 1 was unpreserved and labeled group 1 as well as [1] control. Portion (2, 3, 4 and 5) were mixed with 5 g of salt, garlic, ginger and cloves for 5 minutes and they were labeled preserved. The samples were smoked and separated into group according to the methods described by Omojowo and Ibitoye [26]. The smoked fish samples were allowed to cool down and stored in different clean plastics. The smoked fish samples were subjected to analysis after two, four, six and eight weeks of storage.

Microbiological Examination

Total viable count (TVC), Total fungi count and Total Coliform count (TCC) were recorded according to the research work methods described by Olusegun and Jacob [27]. Bergey's manual was used to identify the microorganisms after the biochemical test.

Proximate Composition of the Samples

Crude protein, ash contents, Moisture contents, fat contents, Crude fibre and Dry matter were examined as per AOAC [28].

Sensory Evaluation

Sensory evaluation was investigated according to the method of Afolabi et al. [29]. In which a total number of 10 panelists, who were selected randomly, assessed the quality of the fish samples. The panelists [2] were rated the samples on a scale ranging from 1-5 by using 5 point hedonic scale of (1=dislike much, 2=dislike, 3=neither like nor dislike, 4=like and 5=like much). The sensory qualities assessed were Colour, Flavour, Texture, Taste and Overall acceptability.

Statistical Analysis

The data generated were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0 and subjected to one way analysis of variance (ANOVA) and Duncan multiple range tests (DMRT) were carried out to test for [5] significance

Results

The results obtained from statistical analysis on microbial analysis of freshly and smoked tilapia fish samples are showed in **Table 1** and the microorganisms isolated from the samples were presented in **Table 2**. **Table 3** shows the results of the mean values of the organoleptic attributes of freshly sample (smoked tilapia) fish before and after storage while the results on average values of proximate composition of freshly smoked tilapia fish samples before and after storage treated with group 1 and 2 are presented in **Table 4**.

To Calculate Colony forming Unit (CFU): CFU/g or ml = $\frac{\text{No of colonies on plates X No of ml plated}}{\text{Dilution factor}}$

DISCUSSION

Microbial Load Counts of the Samples

As shown in **Table 1**, the numbers of microbial colonies formed by each sample was obtained. The microbial load varied significantly (P<0.05) among the five samples (untreated/control, salt, ginger, clove and garlic). TVC at fresh state of the samples, the salt sample recorded higher value of ($9.0 \times 10_3$), followed by unsalted sample of (7.5×10^3)and the least value recorded in garlic sample of (5.0×10^3) while ginger and clove sample recorded (zero/no growth). In the second week, the TVC recorded higher value of (2.5×10^4) in unsalted sample, followed by clove sample of (9.1×10^3) and the least value recorded in ginger sample of (2.4×10^3) while salt and garlic sample recorded (zero/no growth). In the fourth week, the TVC recorded higher value of (5.0×10^4) in clove sample, followed by garlic and unsalted sample of (3.9×10^4) and (2.9×10^4) respectively and the least value recorded in ginger sample of (5.5×10^3) while salt sample recorded (zero/no growth). In the sixth week, the TVC recorded higher value of (7.5×10^4) in salt sample, followed by ginger sample of (1.7×10^4) and the clove and garlic sample recorded (zero/no growth), at the end of the sixth week of storage, unsalted sample got spoilt and no count could not be made again. In the eighth week, the TVC recorded higher value of (3.5×10^5) in salt sample while (zero/no growth) was recorded in ginger, clove and garlic sample and no record was recorded in unsalted sample. According to the International Commission on Microbiology Safety for Food and for good fish product [30], the total viable count (TVC) [8] results for all the 5 samples for this study are below the maximum bacteria count of 5×10^5 cfu and it was shown that bacteria species were presented in all the 5 samples **(Table 2)**. The results obtained were similar to [8] those observed by Olusegun and Jacob [31].

Table 1. Microbial plate counts of preserved and unpreserved smoked tilapia fish
KEY: NG-No growth; TVC-Total viable count; TCC-Total coliform count;
TFC-Total fungal count; SPLT-Spoilt

WEEK 0	TVC (Cfu/g)	TFC (Cfu/g)	TCC (Cfu/g)
Unpreserved	7.5×10^3	9.5×10^3	5.5×10^3
Salt	9.0×10^3	6.1×10^3	3.1×10^4
Ginger	NG	6.5×10^3	NG
Clove	NG	1.1×10^4	NG
Garlic	5.0×10^3	5.0×10^3	NG
WEEK 2			
Unpreserved	2.5×10^4	5.5×10^6	4.2×10^5
Salt	NG	5.1×10^5	3.9×10^4
Ginger	2.4×10^3	4.3×10^4	2.0×10^3
Clove	9.1×10^3	3.9×10^5	5.0×10^4
Ginger	NG	5.4×10^4	NG
WEEK 4			
Unpreserved	2.9×10^4	SPLT	9.0×10^6
Salt	NG	1.4×10^5	NG
Ginger	5.5×10^3	5.0×10^4	NG
Clove	5.0×10^4	2.0×10^4	NG
Garlic	3.9×10^4	5.9×10^4	NG
WEEK 6			
Untreated	SPLT	SPLT	SPLT
Salt	7.5×10^4	3.5×10^5	NG
Ginger	1.7×10^4	5.5×10^4	2.8×10^3
Clove	NG	4.1×10^4	NG
Garlic	NG	5.3×10^5	NG
WEEK 8			
Untreated	SPLT	SPLT	SPLT
Salt	3.5×10^5	NG	NG
Ginger	NG	NG	NG
Clove	NG	4.4×10^5	NG
Garlic	NG	NG	NG

The TFC at fresh state of the samples recorded higher value of (1.1×10^4) in clove sample, followed by unpreserved, ginger, salt and garlic sample of (9.5×10^3), (6.5×10^3), (6.1×10^3) and (5.0×10^3), respectively. In the second week, the TFC recorded higher value of (5.5×10^6) in unsalted sample, followed by salt, clove, garlic and ginger sample of (5.1×10^5), (3.9×10^5), (5.4×10^4), and (4.3×10^4) respectively. In the fourth week, the TFC recorded higher value of (1.4×10^5) in salted sample, followed by garlic, ginger and clove sample of (5.9×10^4), (5.0×10^4) and (2.0×10^4) respectively. While unpreserved sample got spoilt at the week four of storage and the count could not be made again. In the sixth week, the TFC recorded higher value of (5.3×10^5)

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Table 2. Microorganisms isolated from freshly smoked tilapia fish samples

Fish sample	Microorganism (bacteria and fungi species)
Untreated	*Bacillus cereus*, *Klebssiella* spp, *Bacillus Subtilis*, *Proteus mirabilis*, 4 bacteria + 4 fungi species *Nigrospora* spp, *Aspergillus* spp, *Aspergillus flavus*, *penicillium* spp
Salt	*Bacillus cereus*, *Klebssiella* spp, 2 bacteria+2 fungi species *Nigrospora* spp, *Aspergillus* spp
Ginger	*Bacillus subtilis*, *Proteus mirabilis*, *Klebssiella* spp, 3 bacteria+2 fungi species *Aspergillus*, fumigates, mucor
Clove	*Bacillus subtilis*, *Klebssiella* spp, 2 bacteria+2 fungi species *Aspergillus flavus*, *penicillium* spp
Garlic	*Bacillus cereus*, *Bacillus* spp, 2 bacteria+1 fungi species *Penicillum* spp

in garlic sample, followed by salt, ginger and clove sample of (3.5×10^5), (5.5×10^4)and (4.1×10^4) respectively. While no record was recorded in unpreserved sample. In the eighth week, the TFC was only recorded in clove sample of (4.4×10^5) and (zero/no growth) was recorded in salt, ginger and garlic while no recorded was recorded in unpreserved sample. It shows that fungi species were presented in all the 5 samples (**Table 2**). As seen in this study during storage of samples (smoked fish) there was significant ($P<0.05$) increase in the fungi count with length of storage. This result is similar to the work of Oyebamiji et al. and Wogu et al. [32-33]. The occurrence of fungi may be due to the difference in the chemical composition and to which different moulds react differently of the fish species [34,35]. The TCC at fresh state of the samples recorded higher value of (1.1×10^4) in salt sample and the least value of (5.5×10^3) in unpreserved sample, while (zero/no growth) was recorded in ginger, clove and garlic sample. In the second week, the TCC recorded higher value of (4.2×10^5) in unpreserved sample, followed by clove, salt and ginger sample of (5.0×10^4), (3.9×10^4) and (2.0×10^3) respectively. While garlic sample recorded (zero/no growth). In fourth week, only unpreserved sample was recorded a value of (9.0×10^6) while other samples recorded (zero/no growth). In sixth week, only ginger sample was recorded a value of (2.8×10^3) while salt, clove and garlic samples recorded (zero/no growth) and unpreserved sample got spoilt at the week six of storage and no count could not be made again. In eighth week, all the samples recorded (zero/no growth) except unpreserved sample which have been spoilt at week six of storage. The increased in (TCC) of some fresh fish samples which later generally dropped significantly ($P<0.05$) after 8 weeks of storage may be due to presence of water. These results were similar to the results observed by Olusegun and Jacob [31].

In **Table 3**, the quality of the smoked tilapia fish samples both (group 1 and 2) was evaluated immediately after smoking and storage for 2nd, 4th, 6th and 8th week on colour, flavour, texture, taste and overall acceptability. The overall score was given to both (group 1 and 2) using a hedonic scale of 1- 5 where fish scoring less than 2.0 being regarded as unacceptable while above 2.0 being regarded as acceptable. From the result, the scores for the overall acceptability of freshly smoked tilapia fish (3) were recorded as follows: 2.1 3.3 3.4 3.6 4.5 =5.0 while on the 2nd, 4th, 6th and 8th week the trends were 1.7 2.1 2.3 2.9 3.7=5.0. The results of all the treatments were above 2.0 shows that they are all acceptable by the consumers except the untreated sample which had value less than 2. These results were consistent to the work of Omojowo et al. [36].

Table 3. Mean values of the organoleptic attributes of freshly smoked tilapia fish before storage treated with group 1 and 2
N.B Group 1=sample untreated; Group 2=sample mixed with salt/ginger/clove/garlic

Fish sample	Colour	Flavour	Texture	Taste	Overall acceptability
Untreated (control)	3.5	3.8	4.1	3.7	2.1
Salted	4.8	4.1	4.3	4.7	4.5
Ginger	4	3.7	3.6	3.2	4
Clove	4,1	4.3	3.7	3.3	3.6
Garlic	3.9	4.3	4	3.3	3.3
Mean values of the organoleptic attributes of freshly smoked tilapia fish after storage for 2, 4, 6 and 8 week treated with group 1 and 2					
Untreated (control)	2	1.7	1.3	1	1.7
Salted	4.1	4.3	3.8	3.3	3.7
Ginger	4.5	3.6	3.1	2.9	2.9
Clove	4.7	4	3.6	3	2.1
Garlic	4.6	3.9	3.5	3	2.3

In the **Table 4**, it was shown that proximate composition was increased in crude protein, fat content, ash content and dry matter while there was a rapid moisture and crude fibre reduction of freshly smoked tilapia fish treated with group 1 and 2. These results fall within the range obtained by the earlier study of Idah and Nwankwo [34] which obtained the crude protein rises to 48.87, 49.40 and 64.90% after 15 h of smoke-drying at 50, 60 and 70°C, respectively. This result is in agreement with earlier studies of [35-40] which all observed that smoking/drying increases crude protein, crude lipid, crude fibre and ash content of fish and meat products. It is observed that when there is increase in protein contents there will be decrease in moisture content [28].

CONCLUSION

One of the most common local/natural food preservatives is salt, which tends to improve the shelf life of smoked fish by reducing the number of microbial load on the samples which could have been agent(s) of deterioration or spoilage to the sample

and reduce the shelf life. Shelf life is the period of time in which the food product will remain safe or avoid to get spoilt for a particular period of time, be certain to retain desired sensory attributes, chemical and physical microbiology characteristics.

Table 4. Average values of proximate composition of freshly smoked tilapia fish samples before and after storage treated with group 1 and 2 N.B Group 1=sample unpreserved; Group 2=sample mixed with salt/ginger/clove/garlic

Smoked tilapia fish Sample	% crude protein	% ash content	% fat content	% crude fiber	% dry matter	% moisture content
Untreated	20.32	17.08	4.54	1.79	65.17	75.11
Salt	62.96	19.17	5.8	0.9	73.21	26.79
Ginger	64.75	20.49	7.1	0.92	76.7	23.3
Clove	63.11	18.25	5.6	0.95	72.08	27.92
Garlic	63.52	18.8	6.1	1.02	72.87	27.13

Results of this study are significant because most of these natural preservatives are medicinal which have no effect and add to the flavor and seasoning for some foods and they also act as antimicrobial agents by killing microbes that present in the food. Salt may have little effects on human health and some patients are advised by their medical doctor to be mindful of their salt intake. This work shows that some natural preservatives can serve as substitute for salt in food preservation; it may be due to the physical and chemical properties of the preservatives effects which hinder autolysis and slow down the protein break down of smoked fish samples. With the results of this work, it is recommended that any one of the natural preservatives used in this work can serve as replacement for salt especially ginger sample which had highest percentage of protein and also preserved the smoked tilapia fish for 8 weeks of storage.

Although many researchers have done a lot of work on using salt to preserve smoked fish like the work of Omojowo et al. [41-43]. So therefore, further research work should be carried out on the uses of natural preservatives to preserve smoked fishes [44-47].

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