

ANTIOXIDANT AND ANTIFUNGAL EFFECT OF FRESH GINGER (Zingiber officinale) ON THE SHELF-LIFE OF SMOKED CATFISH

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ABSTRACT

The antioxidant and antifungal effects of ginger paste extracted from fresh ginger on smoked catfish were examined for a 21-day storage period at room temperature (25-30°C). The ginger paste was extracted from fresh ginger by scrapping off the outer coat, ground and blended. Eighty fish samples were gutted and washed thoroughly after which they were divided into five groups of sixteen. Each group was spiced with ginger paste at 10g, 20g, 30g and 40g respectively while the control batch was not spiced. The fishes were then smoked in a smoking kiln for 8hrs. The Superoxide dismutase activity and fungal isolation analyses were carried out to investigate the antioxidant and antifungal effect of ginger paste on the fish samples. The lowest superoxide dismutase and peroxide values (0.64 µ/mg and 7.483 mEq/kg respectively) were recorded in the group of catfish spiced with 40g of ginger (at day 21) and control respectively while the highest Superoxide Dismutase (1.92 μ/mg) and Peroxide (9.141 mEq/kg) values were recorded in the group of catfish spiced with 30g of ginger at Day 21. Mucor circinelloides, Rhizopus stolonifer and Aspergillus niger were isolated from the spiced catfish samples The result revealed that catfish samples spiced with ginger paste had lowest mould count compared with the control group. Recommendation was made for the use of 30-40g of ginger to spice 1kg of catfish in order to reduce microbial growth on them.

Key words: Antioxidant, antifungal, ginger, shelf-life, smoked and catfish

INTRODUCTION

Fish constitutes a very important component of diet for many people and often provides nutrients for a healthy living. Fish serves as a principal source of dietary protein which is very inexpensive in relation to other protein foods (Fawole *et al.*, 2007). Fish is known to contain a very high quality of fats and oil and fish oil is very high in polyunsaturated fatty acid, which is very important in lowering blood cholesterol level (Larsen *et al.*, 2007. The fish oil, on the other hand contains fat-

soluble vitamins. Fish is also a very good source of thiamine and riboflavin and contains minerals, phospholipids sterols, enzymes, hormones, hydrocarbons and pigments (Usydus *et al.*, 2009). The fish muscle contains four basic nutrients in varying proportions; water 70-80%, protein 16-25%, lipids 1-5% and vitamins (Clucas, 1982) which makes it less tough and more digestible compared to beef, chicken and mutton. Fish has high level of essential sulfur-containing amino acids such as



cysteine, methionine and lysine which are limited in some legumes and most cereal diets (Paul and Southgate, 1978). It is the characteristics of fish as a cheap source of animal protein which is now evident throughout the world that makes it an excellent component of human diet (Usydus et al., 2009). Fish protein now takes precedence over other protein of animal origin, compares favorably with milk, egg and is used for its amino acid composition. It is this quality that makes fish protein to be practically indispensable to developing countries such as Nigeria for supplementation where the staple diet or food consist primarily starchy foods (Idris et al., 2010). Shelf life-promoting strategy for fish products prior to processing involves curling with preservatives (Ravishankar and Juneja, 2000). Treatment of fish with spices prior to smoking will prolong the shelf life of the products during storage. Traditional preserving methods such salting, as fermenting, drying and smoking are still widely accepted around the world because of their specific taste and aroma. However, these methods still differ from country to country and within each country in the amounts of additive, percentage of salt or vinegar and maturing temperatures (Egba et al., 2010). A lot of farmed catfish are turned out annually to provide the much-needed animal protein which is in the past obtained from beef (Afzal et al., 2001). Beef meat has been implicated in the coronary disease and most people have avoided consuming it (Talat et al., 2006). Fish is an extremely perishable commodity spoiling soon after death due to enzymatic and microbial actions. Some factors responsible for this include the

prevailing high temperatures in Nigeria and the facilities for processing; storing and distributing the fish caught are frequently inadequate (Talat et al., 2006). There is therefore enormous waste through spoilage of both fresh and dried fish (Adeyeye, 2009; Oluwaniyi and Dosumu, 2010). One of the greatest challenges to the catfish industry is the fact that if not well preserved, fish deteriorates very fast (Kumolu-Johnson and Ndimele, 2011). Only a negligible proportion of fresh fish caught in lakes and rivers in Nigeria is sold fresh. Great portion is preserved by smoking and sun-drying (Ikeme and Blandary, 2001). These deterioration and spoilage are brought about by the action of microbial proliferation and lipid oxidation. The effect of lipid oxidation on fish is the reduction of its nutritional quality which affects its marketability (Sallam et al., 2004). Apart from nutritional loss, the presence of micro-organisms in fish can lead to massive economic loss (Kumolu-Johnson and Ndimele, 2011).

Some processing methods have been used over the years to extend the shelf-life of fish in Nigeria and other part of the world which includes chilling, freezing, salting, drying, canning and smoking (Akintola and Bakare, 2011). Each of the listed processing methods has its own advantages and disadvantages which affects its usage in different parts of the world (Eyo, 2000).

Heat generated as a result of smoking removes water, inhibits bacterial growth, retard enzymatic action, imparts aroma, taste and color on processed fish but its quality can deteriorate during storage due to lipid oxidation and microbial growth (Eyo, 2000). Inspite of the modest success of fish smoking



especially in terms of increasing the shelf-life of fish and nutritional quality of fish, the volume of post-harvest losses especially in sub-Saharan Africa is quite worrisome. It becomes a thing of necessity to put some measures in place so as to prevent lipid oxidation and further deter microbial proliferation (Akram et al., 2011). Synthetic antioxidants like Butylated Hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA) have been effective in combating and controlling rancidity (Martinez-Tome et al., 2001). However, these synthetic antioxidants have been withdrawn from the market because of undesirable effect they have on the enzymes of the liver and lung of pigs (Inatani et al., 1982). Spices are edible plant materials that have antioxidative, antiseptic and bacterio-static properties (Eyo, 2001). These spices are added to food like fish and meat to delay the onset of rancidity and microbial proliferation (Eyo, 2001). Another function of spices is that they act as seasoning to food and add to their flavor (Lafon et al., 1984). Ginger contains essential oil and is used for treatment of fungi, protozoan, viral and bacterial diseases (Adodo, 2002). Fish is known to contain a very high quality of fats and oil and fish oil is very high in polyunsaturated fatty acid, which is very important in lowering blood cholesterol level (Larsen et al., 2007. The fish oil, on the other hand contains the fat-soluble vitamins. Fish is also a very good source of thiamine and riboflavin and contains minerals. phospholipids sterols, enzymes, hormones, hydrocarbons and pigments (Usydus et al., 2009). Ginger is used for the treatment of fungi, protozoan, viral and bacterial disease (Adodo, 2002). Ginger as a spice has a

geographical spread that covers every part of the globe and it is consumed whole as a delicacy, used in traditional oriental medicine, or as spice in foods such as fish (Onyeagba et al., 2004; Abdul et al., 2008 and Akram et al., 2011). Ginger contains a spectrum of biologically active compounds, such as curcumin, 6-gingerol, 6-shagoals, zingiberene, bisabolene and several types of lipids on it, and has the properties of being pungent and a stimulant. These compounds are responsible for the unique aroma and flavor of ginger, and account for about 1-3% of the weight of fresh ginger (Akram et al., 2011).

The aim of this study was to determine the antioxidant and antifungal effect of fresh ginger on the shelf-life of smoked catfish (*Clarias gariepinus*)

MATERIALS AND METHOD Study Location

work This was conducted at the Microbiology Laboratory and Industial Chemistry Laboratory of the University of Ilorin, Kwara State, Nigeria on latitude 8°30` and 8°50'N and longitude 4°20' and 4°35'E of the equator. The daily average temperatures are low in January with 25 °C, rise in May to 27.5 °C and lowest in September with 22.5 °C (Kwara State Ministry of Environment, 2017).

SAMPLE COLLECTION

Eighty juveniles of *Clarias gariepinus* with average weight of 330g were obtained from Ifesowapo Fish Farm in Ilorin metropolis and were transported to the Central Laboratory, Faculty of Agriculture, University of Ilorin for storage inside a big deep freezer. Bulky fresh gingers were also bought from a local



market at Tanke, Oke-Odo, Ilorin for the study.

FISH HANDLING AND PROCESSING

Fish were thawed, eviscerated and prepared into "butterfly cut" according to the method of Roger et al. (1975). The fish were washed, brined by dipping in 15% sodium chloride for 3minutes and then drained after which they were then divided into five groups with each group containing sixteen fishes. The outer coats of fresh gingers were scraped off and cleaned. They were later grounded with a mortar after which they were blended with kitchen blender to form ginger paste. The paste was then added to each of the batches of fish in form of spice mixture at different concentration levels (10, 20, 30 and 40g of ginger per kg of fish) and control with 0.00g of ginger. The fish were smoked in a smoking kiln with charcoal for 8hrs and the smoked samples were cooled, packaged with aluminum foil and then stored at ambient temperature of 25°C-30°C in the laboratory for 21days.

CHEMICAL ANALYSIS

Peroxide evaluation

The oxidative stability of smoked catfish was measured using titrimetric method to determine the amount of peroxide and hydro peroxide group (the initial product of lipid oxidation) respectively using the method of AOAC (1995).

Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity was observed following its ability to inhibit auto-oxidation of epinephrine estimated by increase in absorbance at 480nm. The reaction mixture contained 2.95ml of 0.05M Sodium Carbonate buffer;pH 10.2, 0.02ml of tissue

homogenate and 0.03ml of epinephrine. 0.005N HCL was used to initiate the reaction. The blank reaction was determined. The absorbance was taken at 480nm for 5min using the method of Sun and Zigmas (1978).

Microbiological analysis

All materials used were sterilized to prevent microbial contamination. The work bench was sterilized using 70% alcohol, all media and contaminated materials were autoclaved at 121°C for 15min. Glassware were sterilized in hot air oven at 170°C for 2hours. Petri-dishes were bought sterile. Aseptic techniques were used to prevent contamination

Preparation of Culture Media

The culture media, Potato Dextrose Agar was used for the isolation of fungi smoked catfish. The Potato Dextrose Agar was prepared using the method of Abubakar (2016). The PDA was prepared as follows:

Preparation of Potato Dextrose Agar (PDA)

Peeled-sliced potatoes 200g

Dextrose (glucose) 10-20g

Agar 12-15g

Distilled water1liter

Sliced potato was added to water and allowed to simmer for 30-60minutes after which it was filtered through layers of cheese-cloth. Agar and other ingredients were added to the filtrate before autoclaving. To discourage bacterial contamination, 100mg of streptomycin sulphate, prepared as stock solution in sterile distilled water, was added to 1litre of autoclaved medium under sterile hood at 45°C before pouring into plates. With this addition, the medium is known as PDAs.



Fungal Isolation counts

The fungal isolation and counts were done using pour plate technique. 1.0g of the smoked fish was grinded in a sterilized mortar to a powdery form, then 1ml of sterile distilled water was added to make a stock solution then serial dilution was carried out and then 1ml of the second and third diluent was introduced into a sterile petri-dish, cool molten PDA was poured into the plate and it was swirled clockwise and anticlockwise to allow for even distribution. The plates were incubated at room temperature (26-27°C) for 3-5days after which the plates were observed for fungi growth which were counted. The fungi isolated were further purified by subculturing them in a PDA to obtain a pure fresh culture.

Characterization of Fungi

Fungi identification carried was macroscopically by observing the colonial morphology of each isolate. Features such as surface texture, color shape, and pigmentation of the mycelium were observed, each isolate was then observed microscopically using the wet mount technique. One drop of distilled water was

placed on a glass slide, a portion of mycelium was picked and dropped on the glass slide and then teased with the aid of an inoculating wire needle. Two drops of methylene blue were added, and covered with a cover slip then observed under x100 objective lens. Hyphal nature characteristics and disposition of mature fruiting structures were revealed. Morphological structures were identified using Descriptive Manuals (Alexopoulos and Mims, 1979; Beech et al., 1986 and Kavanagh, 2005).

STATISTICAL ANALYSIS

Data were analyzed with Microsoft Excel and using Statistic Product for Social Sciences (SPSS) version 16.0 for windows. Statistical difference between means were compared using Turkey HSD for p<0.05.

RESULTS

Chemical Analysis Result

Table 1 shows the chemical analysis of superoxide Dismutase activity and Peroxide Values on the fish samples (0g, 10g, 20g, 30g and 40g) on ginger respectively.

TABLE 1: SUPEROXIDE DISMUTASE AND PEROXIDE CONCENTRATION IN FISH SAMPLES SPICED WITH GINGER

			Superoxide Dismutase (µ/mg)		PV (mEq/kg)			
Samples	Day 1			Day	21			
T1 (µ/mg)	1.14	1.10	1.11	1.68	1.67	1.69	8.921	8.923
T2 (μ /mg)	1.16	1.15	1.14	1.69	1.70	1.72	8.842	9.844
T3 (μ /mg)	1.21	1.20	1.21	1.92	2.01	2.00	9.140	9.141
T4 (μ /mg)	0.64	0.64	0.64	1.21	1.20	1.20	8.672	8.672
Control	0.67	0.68	0.66	1.18	1.17	1.18	7.480	7.483

KEY:

T1-T4: Ginger concentration per kg of fish from 10g-40g respectively;

Control: 0g of ginger;

PV: peroxide value taken for each treatment level.



TABLE 2: MEAN VALUE OF SUPEROXIDE DISMUTASE AND PEROXIDE VALUE USED FOR CATFISH SPICED WITH GINGER FOR A 21-DAY STORAGE PERIOD

Treatment	Day 1 (SOD)	Day 21 (SOD)	PV
T1	1.117 ± 0.02^{c}	1.680 ± 0.01^{b}	8.922 ± 0.00^{b}
T2	1.150 ± 0.01^{b}	1.703 ± 0.02^{b}	8.843 ± 0.00^{c}
T3	1.207 ± 0.01^a	1.977 ± 0.49^a	9.141 ± 0.00^{a}
T4	$0.640\pm0.00^{\rm e}$	1.203 ± 0.01^{c}	8.672 ± 0.00^{d}
Control	0.670 ± 0.01^{d}	1.177 ± 0.01^{c}	7.482 ± 0.00^{e}

Mean value with different superscript along the column are significantly different at (p<0.05)

KEY: T1-T4 represents ginger concentration per kg of fish from 10g-40g respectively

Control: 0g of ginger;

SOD: Superoxide Dismutase analysis recorded for each treatment;

PV: Peroxide value taken for each treatment level.

The table depicts the mean values that were analyzed from the readings taken from the laboratory in the course of a 21-day storage period.

The mean values obtained from Day 1 of the Superoxide Dismutase activity shows that all the treatments from T1-T4 and control were significantly different from each other. The lowest value was recorded at 40g of ginger (T4) while the highest recorded value was at 30g of ginger (T3).

p=0.000 was recorded for Day 1 which means p<0.05 is accepted for Day 1 Superoxide Dismutase activity. For Day 21 however, SOD activity recorded at T1 and T2 were not significantly different from each other but were both significantly different to the 3 remaining treatments. So also, T4 and Control were not significantly different from each other but were both significantly different from each other but were both significantly different from other treatments. T3, ginger at 30g was significantly different from other treatments. The highest value recorded for Day 21 was at T3 while the lowest was the

Control. p=0.000 was recorded for Day 21 which connotes that p<0.05 was accepted for Day 21 Superoxide Dismutase activity.

For peroxide value taken on the 21st day of the storage, the values recorded were all significantly different from each other. Highest value was recorded at T3 while the lowest was at Control. So also, p value=0.000 was recorded which means there was significant different (p<0.05) in the peroxide values taken.

There was a general increase in the SOD activity of the ginger treated samples and untreated sample (Control) during the storage period but the values recorded for the ginger treated samples were higher than that of the control except for the T4 value recorded at Day 1 which had a lesser value compared with the Control recorded on the same day

MICROBIOLOGICAL ANALYSIS RESULT

The Table 3 below shows the fungi analysis from the fish samples.



TABLE 3:FUNGI ANALYSIS (COUNT) AT DIFFERENT LEVELS OF TREATED FISH SAMPLES AND CONTROL

S/n	Sample Code (g/kg)	Fungi Count	(CFU/ml)	
Day 1	Control	ND	ND	ND
	T10	9.0×10^3	4.0×10^4	2.0×10^5
	T20	1.0×10^4	2.0×10^4	1.0×10^5
	T30	8.0×10^3	2.0×10^4	1.0×10^5
	T40	1.0×10^3	ND	ND
Day 7	Control	2.0×10^4	1.4×10^5	1.0×10^6
	T10	7.0×10^3	2.0×10^4	1.0×10^5
	T20	6.0×10^3	3.0×10^4	2.0×10^5
	T30	3.0×10^3	1.0×10^4	ND
	T40	ND	ND	ND
Day 21	Control	3.6×10^4	1.9×10^5	1.2×10^6
	T10	1.3×10^4	5.0×10^4	3.0×10^5
	T20	2.0×10^3	ND	ND
	T30	1.0×10^3	ND	ND
	T40	ND	ND	ND

KEY:

ND: No growth

Control: ginger concentration at 0g per kg of fish T10: ginger concentration at 10g per kg of fish T20: ginger concentration at 20g per kg of fish T30:ginger concentration at 30g per kg of fish T40: ginger concentration at 40g per kg of fish

TABLE 4: MEAN VALUE OF FUNGI COUNTS ON GINGER TREATED CATFISH AND CONTROL FOR 21DAY STORAGE PERIOD

Treatment	Day 1	Day 7	Day 21
Control	0.000 ± 0.00^{b}	3.816±1.03 ^a	3.765±0.76 ^a
T10	3.619 ± 0.67^{a}	3.049 ± 0.23^{a}	3.763 ± 0.68^a
T20	3.434 ± 0.51^a	3.519 ± 0.76^{a}	0.767 ± 1.33^{b}
T30	3.068 ± 0.21^{a}	1.826 ± 1.60^{b}	0.667 ± 1.16^{b}
T40	0.667 ± 1.16^{b}	0.000 ± 0.00^{c}	0.000 ± 0.00^{b}

Mean value with different superscripts along the rows are significantly different at (p<0.05)



The Table 4 revealed the mean fungi counts from both the treated fish samples and the control samples for 21-day storage period.

The values for Day 1 showed that there was no significant difference between control and T40 but they were significantly different in comparison to other treatment groups. T10-T30 groups were also not significantly different from each other but were significantly different to the Control and T40. The highest mean fungi count was recorded at T10 while the lowest fungi count was that of control which recorded no growth. The p value for the Day 1 fungi count was taken as 0.000 which means they were significantly different (p<0.05).

On Day 7, fungi counts were significantly different between the control samples and treated samples (T30 and T40 mainly). Therewas no significant difference among T10, T20 and the control. T30 and T40 were significantly different to the other samples. The highest fungi count mean at Day 7 was that of control (untreated) sample while the lowest count was the T40 samples which had no growth which means there was

significant different among the samples (p<0.05).

On Day 21, there was no significant difference between the Control and T10 but there were significant different between the control and other treated groups. So also, T20, T30 and T40 were not significantly different to each other but were significantly different compared to the control and T10. The highest mean value was recorded in the Control whereas the lowest value was recorded in the T40. P value was taken at 0.000 which indicates that there was a significant different between the samples' treatment (p<0.05).

Three genera of fungi were isolated (*Mucor circinelloides*, *Rhizopus stolonifer* and *Aspergillus niger*) from the fish samples (Plates 1, 2 and 3). The results on macroculture and microscopy characteristics of these fungi are tabulated in Table 5. Results showed that the effect of *Mucor circinelloides* on the fish samples were similar to the report of the work of Ogunniran (2019).



TABLE 5: MACROCULTURE AND MICROSCOPY CHARACTERISTICS OF FUNGI ISOLATED FROM THE SAMPLED FISH

Fungi Isolates	Macroculture	Microscopy
Mucor circinelloides	Growth: Quick covering the agar surface in just	Hyphae: thick, non-septate.
	few days. Growth form: long-fibred, rough woolly network of hyphae Colour: Pale greyish brown	Sporangiophore: ramified and spherical at the end. Conidia: elliptical, contained in large numbers in the sporangia
Rhizopus stolonifer	Growth: the colonies grew rapidly covering the whole plate Growth form: body of branching	Hyphae: thick, non-septate; Sporangiophores: globose.
	mycelia composed of three types of hyphae Colour: isolate colonies were initially white but turned brown eventually.	Conidia:elliptical
Aspergillus niger.	Growth: recognizable within few days. Growth form: velvety to flaky surface due to marked sporulation	Hyphae: septate Conidiophores: borne literally on the hyphae, non -septate; numerous sterigmata proceed from the apical club-shaped swellings (head-shaped fructification organs);
	Color: colorless and later turned black due to formation of conidia.	Conidia: borne in chains on the sterigmata.

PHOTOMICROGRAPHS OF FUNGI ISOLATED FROM THE FISH SAMPLES

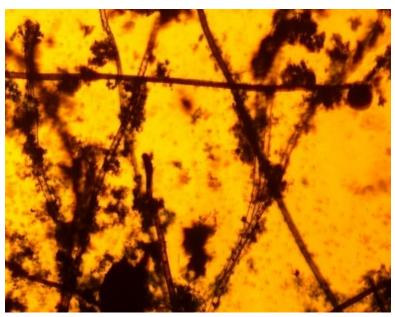


PLATE1: PHOTOMICROGRAPH OF MUCOR CIRCINELLOIDES FROM SMOKED CATFISH (MG X800)



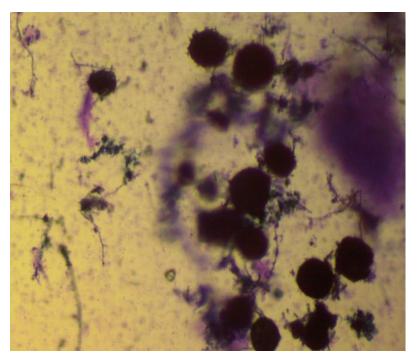


PLATE 2: PHOTOMICROGRAPH OF $\it RHIZOPUS$ $\it STOLONIFER$ FROM SMOKED CATFISH (MG X800)

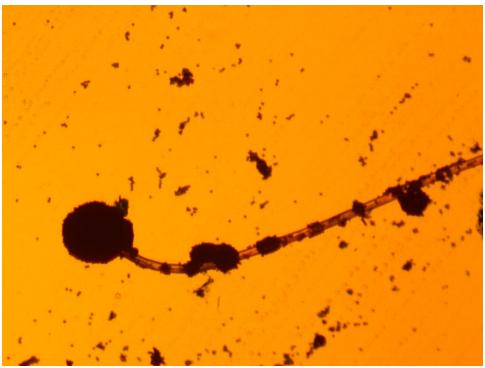


PLATE 3: PHOTOMICROGRAPH OF $ASPERGILLUS\ NIGER$ FROM SMOKED CATFISH (MG X800)



DISCUSSION

The results of this study show the effect of the ginger on the treated catfish samples. In this study, the highest level of Superoxide Dismutase recorded on 21 day for T4 (40g of ginger) shows that the ginger was effective in retarding the oxidative rancidity in the treated fish at this level. This agrees with the work of Kumolu-Johnson and Ndimele (2011) in which fresh ginger was used to increase the shelf-life of hot smoked catfish. It was noticed that the control which has 0g of ginger added to it had lower value after the SOD activity was analyzed whereas all the ginger treated sampleshad higher SOD values except for the 40g of ginger on the first day. Untreated sample also had a low value because of the effect of smoke on fish which has its own antioxidant effect on it. Relatively low oxidation values recorded for the treated samples justify the effects of antioxidants in the samples. This is an indication of ginger being a good antioxidant. This is in line with the work of Ikpeme *et al*. (2016) who reported the effectiveness of ginger and Watermelon in reducing oxidative stress in Rats model. The mean peroxide value of 25mEq/kg of active 0₂/kg recordedin this study fall within the values acceptable for fatty foods. This also agreed with the result of the work of Kumolu et al., (2013) who worked on antioxidant effect of ginger on smoked fish. The effectiveness of ginger as an antioxidant is directly related to the concentration of the ginger. This agreed with the work of Saito et al., (1976) and Lee et al., (1986) who used spice as antioxidant and made it relatively direct to the spices' concentration. The difference in the fungi growth on smoked catfish sample treated

with different ginger concentrations was significant (p<0.05). This therefore showed that fresh ginger was very effective in the reduction of fungal growth on the smoked fish stored at an ambient temperature of 25-30°C for 21 days. Antifungal effects of ginger might be due to its chemical properties which agreed with the work of Tagoe et al. (2011) who studied the antifungal properties of ginger, onion and garlic on Aspergillus niger, Aspergillus flavus and Cladosporum herbarum. It is also in agreement with Ogunniran (2019) who isolated Mucor circinelloides, Rhizopus *stolonifer* and Aspergillus niger from his work on antioxidant and antifungal effect of fresh ginger on the shelf-life of smoked catfish. The steady increase in fungal growth throughout the 21-day storage period observed in the untreated samples revealed the effectiveness of ginger as an antifungal agent. It is also noticed that no fungal growth was recorded in the T40 (40g of ginger) on the 21st day of the analysis. This shows that fungi growth was effectively retarded when 40g of ginger was used for 1kg of smoked catfish. This is in line with the work of Kumolu-Johnson and Ndimele (2011) where ginger was used to reduce microbial load in fish. This also agreed with the work of Negbenebor et al., (1996) who used clove and ginger to reduce fungal growth in smoked fish.This studytherefore affirms that ginger, anatural spice contains antifungal properties which can compare favorably with synthetic antimicrobial agents as reported Omojowoet al., (2008), Omojowoet al., (2009a) and Omojowoet al., (2009b) where citric acid was used as preservatives in smoked catfish.



CONCLUSION

This study revealed that the application of ginger on fish can prolong its shelf life by retarding its oxidative rancidity and fungi growth. It therefore shows theimportance of ginger in retardation of oxidative rancidity and fungal growth on the smoked catfish.

RECOMMENDATION

For proper antifungal sensitivity, it is recommended that 30-40g of fresh ginger should be added to 1kg of catfish before smoking.

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