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Potentials of *Allium Sativum* as antimicrobial agent against fungi and bacterial invasion on smoked dried *Clarias gariepinus*

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Abstract

Freshly caught fish were treated with garlic bulbs extracts at various levels of dilution, 0%(A), 25%(B), 50%(C),75% (D) and then smoked in smoking kilns at uniform temperatures. Smoking temperatures ranged between 100 and 150^{0}C. Products were packaged in labelled sterile paper bags and assessed biweekly for spoilage and organoleptic properties. Results analysis showed that peroxide value was lowest in treatment with 75% garlic (6.09m/Eq/kg) and highest (7.86mEq/kg) in 0% garlic treated. Microbial load reduces with increase concentration of garlic extract in the treatment. Acceptability of the products preserved with garlic was almost uniform for the six weeks in contrast to the 0% treatment which was partially accepted after six weeks. Garlic is therefore an acceptable preservative for *Clarias gariepinus*. especially at 75% concentration.

Keywords: *Allium Sativum*; Bacteria; *Clarias Gariepinus*

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1. Introduction

Fishing activities have been noted to result in wastage as a result of poor preservation techniques employed by fishers. Fish like other agricultural produce is a perishable biomaterial especially in the tropics where high temperature and humidity accelerate spoilage and bio-deterioration of fish which starts immediately after catch (Okonta and Ekelemu, 2005). Fish is subject to post harvest losses ranging from bacterial and autolytic spoilage to other factors. These cause fish to lose its organoleptic qualities, and generally become unacceptable for human consumption. It is the quest to reduce perishability of fish that leads to the processing fish into various products, such as smoked, dried and canned fish, fish cake, fish meal and fish burger. The reduction in losses can only be achieved by systematic improvements in handling, processing, storage and distribution (Clucas, 1998). Some of the techniques employed in fish preservation include removal of water, this can be achieved by air, wind, open sun or solar, heat or freezing; addition of salt. Smoking is by far the most popular preservative method in developing countries because it does not require energy as canning and freezing which is adopted in the developed world. The heat generated removes water, kills and or inactivate microbes while the phenolic compounds from smoke and the salt that is usually added during preparation combined to improve the shelf life of smoked fish to an extent. Smoked fish is a delicacy all over the world because of the taste and flavour which is highly valued by consumers. However, smoked fish is still susceptible to post-smoking contamination due to handling and packaging issues. Though the temperature achieved during hot-smoking could kill some vegetative microorganisms their spores are not killed or destroyed. The spores would normally emerge and in combination with post-smoking contaminants to reduce the shelf life of smoked fish (Vaz-Vello, 2003). While prolonging the shelf life of smoked fish might not be a challenge in the developed world whe.re there is constant and regular supply of good quality energy and refrigeration and freezing can work, it is an issue of serious concern in the developing world. The inefficiency and incessant outage of energy supply in developing country make it inevitable to source for additional preservatives to improve the shelf life of smoked fish.

Apart from sodium chloride that is commonly used to improve taste and or reduced microbial attack on fish, chemical preservatives that are used in food include formalin (Bamti, 2016; NIE, 2017), butylated hydroxyanisole (BHA), butylated hydroxytoluene, tert-butyl hydroquinone, ethoxyquin (Vwioko, 2013), lactate, sorbic acid (Antonia et al., 2008); lactic acid (Fernandes et al., 1998), sodium benzoate (Efiuvwevwere and Ajiboye, 1996). Though these chemicals have been found to be effective against microbes they also post serious health risk to human consumers of the products preserved by them. Many of them have been linked to life threatening illness such as cancer, food poisoning and other health issues (Bamti, 2016 and Vwioko, 2013). Thus, there has been an increasing desire to explore and exploit preservative potentials of common natural spices as alternatives to the synthetic additives.

Many natural food spices among which are the *Alliumeaceae* and *Zingiberaceae* have been found to be medicinal and have antimicrobial properties. Garlic (*Allium sativum*) and onion (*Allium cepa*) are among the important *Allium* species that are widely consumed. Garlic contains alliin which is converted by the enzyme allinase to allicin derivative products (diallyl disulfide, diallyl trisulfide) and ajoene. The Allicin and Ajoene found in garlic essential oils have good antimicrobial and antioxidant activities (Grela, 2006). Garlic was found

to be effective as preservative for fruit juice extract of sour sop (Vwioko, 2013) and tomato puree (Antimicrobial and preservative activities of Allium

sativum and Eugenia aromatica on fresh tomato puree, 2008). This study investigates the efficiency of *Allium sativum* (garlic) as antifungal and antibacterial agent for smoked *Clarias gariepinus*.

2. Methodology

Fresh garlic (*Allium sativum*) rhizomes were properly cleaned, washed, peeled and ground into pulp. The pulp was mixed with water in a ratio of 0g, 300g, 500g and 750g of garlic to 1 litre of water respectively these gave a filtrate concentration of 0% (A), 25% (B), 50% (C),75% (D) the mixtures were then heated to 100^0C and later centrifuged (2200rpm) to remove the filtrate and then cooled to room temperature.

Whole fresh 250g *Clarias gariepinus* fish were obtained from the University Fish Farm, prepared and grouped into four treatments of 9 fish each, each of the groups of fish were soaked for 90minutes in each respective treatment. The whole experimental set up was replicate thrice, hence there were 9 x 3 fish per treatment. The fish were brought out of the water and arranged in separate sieves to drain. The drained fish were then arranged in smoking trays within the smoking kiln for 8hours at temperatures ranging between 100 and 150^0C. The products were packaged in sterile paper bags base on treatment and the number of replicate, kept in the shelves at ambient temperature where they were assessed biweekly for bacterial, fungal, peroxide value after the procedures recommended by AOAC (2005) and organoleptic properties were measured using Hedonic Scale (Hedonic Scale, 2005 & SSP, 2018). Results were analysed statistically using descriptive statistics and One Way Analysis of Variance (ANOVA) with SPSS version 23 for Microsoft windows. Duncan Multiple Range test at 5% level of significance was used to determine the differences in mean of all treatments.

3. Results and Discussion

3.1. Bacterial count

3.1.1. Fish head

The bacteria population on the head of smoked C gariepinus became visible after the second week of smoking. The population increased rapidly in Treatment B, C and D in the second week after which a gradual decrease was observed especially in C. Bacterial population was maintained at 10.00 ± 0.58 x$10^2$$Log_{10}$Cfu/g in specimen D from the second week till the sixth week. The head of fish in D have the lowest population of bacterial than those in A, B and C and there seems to be no evidence of increase in population of bacteria in the treatment (Table 1).

Table 1. Mean population of Bacterial on the head of *C. gariepinus* preserved in various Concentrations of Garlic Extract ($x10^2Log_{10}Cfu/g$)

	WEEKS			
TREATMENT	**WEEK 0**	**WEEK 2**	**WEEK 4**	**WEEK 6**
A	0.00 ± 0.00^a	4.00 ± 0.00^a	20.00 ± 0.00^c	60.00 ± 0.58^c
B	0.00 ± 0.00^a	20.00 ± 0.58^c	20.00 ± 0.58^c	10.00 ± 0.58^b
C	0.00 ± 0.00^a	30.00 ± 0.58^d	20.00 ± 0.58^c	10.00 ± 0.58^b
D	0.00 ± 0.00^a	10.00 ± 0.00^b	10.00 ± 0.58^b	10.00 ± 0.58^b

3.1.2. Fish flesh

Similarly, in treatment D, mean bacterial population in the flesh increased from the first to the second week, there was no visible increase in the number of bacteria seen in the fourth week and the mean population then decreased by the sixth week (10.00 $Log_{10}Cfu/g$), whereas in treatment A there was visible increase as the week progresses to 80.00 $Log_{10}Cfu/g$ by week 6 (Table 2). Bacteria load in the fish head appeared more across the various weeks than in the flesh. This is expectedly so because the head houses the gills which are in constant touch with the natural aquatic environment during feeding and respiration.

Table 2. Mean population of Bacterial on the flesh of *C. gariepinus* preserved in various Concentrations of Garlic Extract ($x10^2Log_{10}Cfu/g$)

	WEEKS			
TREATMENT	**WEEK 0**	**WEEK 2**	**WEEK 4**	**WEEK 6**
A	1.00 ± 0.00	10.00 ± 0.58^b	20.00 ± 0.58^b	80.00 ± 0.58^d
B	0.00 ± 0.00	10.00 ± 0.58^b	10.00 ± 0.58^a	10.00 ± 0.58^b
C	0.00 ± 0.00	0.00 ± 0.00^a	10.00 ± 0.00^a	20.00 ± 0.58^c
D	0.00 ± 0.00	10.00 ± 0.00^a	10.00 ± 0.58^a	0.00 ± 0.00^a

Means with different superscripts are significantly different from one another

3.2. Fungal population

3.2.1. Fish head

Fungal populations were not visible in the first week in all the treatments, the second week witnessed a high population of fungi in treatment A ($30.00\pm0.00\ x10^5Log_{10}Cfu/g$) and D ($40.0000\pm0.00\ x10^5Log_{10}Cfu/g$) than B

and C. However, by the end of the 6th week fungal population reduced with increase concentration of garlic extract with no visible microbe recorded in treatment C and D (Table 3).

Table 3. Mean population of fungi in the head of *C. gariepinus* preserved in various Concentrations Garlic Extract ($x10^2Log_{10}Cfu/g$)

	WEEKS			
TREATMENT	**WEEK 0**	**WEEK 2**	**WEEK 4**	**WEEK 6**
A	0.00 ± 0.00^a	30.00 ± 0.00^d	20.00 ± 0.58^c	40.00 ± 0.58^e
B	0.00 ± 0.00^a	0.00 ± 0.00^a	10.00 ± 0.58^b	10.00 ± 0.00^b
C	0.00 ± 0.00^a	20.00 ± 0.58^c	0.00 ± 0.00^a	0.00 ± 0.00^a
D	0.00 ± 0.00^a	40.00 ± 0.00^e	0.00 ± 0.00^a	0.00 ± 0.00^a

Means with different superscripts are significantly different from one another

3.2.2. Fish flesh

The population of fungi was visible all through the weeks except in week 0, the growth pattern was zigmoid dropping from 20.00 ± 0.00 $x10^2Log_{10}Cfu/g$ in the second week to 10.00 ± 0.00 $x10^2Log_{10}Cfu/g$ and then rose to 20.00 ± 0.00 $x10^2Log_{10}Cfu/g$ by the end of the 6th week. None of the garlic treated fish showed visible presence of fungi by the 6th week (Table 4).

Table 4. Mean population of fungi *C. gariepinus* flesh preserved in various Concentrations Garlic Extract ($x10^2Log_{10}Cfu/g$)

	WEEKS			
TREATMENT	**WEEK 0**	**WEEK 2**	**WEEK 4**	**WEEK 6**
A	0.00 ± 0.00	20.00 ± 0.00^c	10.00 ± 0.58^a	20.00 ± 0.58^c
B	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.58^a	0.00 ± 0.00
C	0.00 ± 0.00	10.00 ± 0.00^a	10.00 ± 0.58^a	0.00 ± 0.00
D	0.00 ± 0.00	20.00 ± 0.00^c	0.00 ± 0.00^a	0.00 ± 0.00

Means with different superscripts are significantly different from one another

The head of the fish have more fungi presence than the flesh as it was observed with the bacteria. The higher population of microbes in the head of fish may not be unconnected with the presence of resident flora in the gills as a result of the constant interaction they have with the fluid from the external environment during respiration and feeding.

Garlic extract significantly hindered the growth of bacteria and fungi in the flesh and head of the smoked *Clarias gariepinus* used in this study. The suppression of microbial population may be connected with the activity of *Allicin* and *ajoe* an active ingredient in garlic (Sudarshan et al., 2010). Similar results were obtained on fruit juice extract of sour sop (Vwioko, 2013) and tomato puree (Adekalu, 2009). There was no significant statistical difference in acceptability of the garlic product with length of preservation.

The peroxide value was lowest (6.09m/Eq/kg) in 75% garlic extract treatment treatment and highest (7.86mEq/kg) in 0%garlic extract treatment (A). This suggests retardation in the development of oxidative rancidity by garlic. This assertion is supported by several authors (Ikeme and Bhandary, 2001; Kumolu-Johnson et al., 2013).

Acceptability of the products preserved with garlic was almost uniform for the six weeks in contrast to the 0% treatment which was partially accepted after six weeks.

4. Conclusions

The study shows that garlic extract could effectively control bacterial and fungal invasion on smoked dried *Clarias gariepinus*. The best concentration was at 75% in a range of 25 -75% concentration. The antimicrobial potency of *Allium sativum* provides an effective and safe preservative for smoked dried *C. gariepinus*. The product also has high acceptability which was almost uniform for the six weeks at ambient temperature in contrast to the untreated ones. Garlic treatment of fish products could be a good additive for preservation of *Clarias gariepinus*.

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