Efficacy of powdered neem leaves (*Azadirachta indica*) as anti fungal agent on smoked dried fillets of African Catfish (Chrysichthys nigrodigitatus)

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Abstract

The efficacy of powdered neem as a preservative agent on the fillets of *Chrysichthys nigrodigitatus* was investigated; in search of alternatives to chemical antimicrobial agents in response to health concerns and economic reasons. Fillets of 50g ± 5 were prepared, treated with 30% brine and hot smoked to constant weight in an enclosed coal oven. Samples were divided into three parts; X was dusted with air dried powdered neem leaves, Y with freshly extracted vegetable oil, and Z was untreated. Products were packaged in airtight polythene containers, stored and monitored for microbial development for 12 weeks. Nutrient quality was determined.

Mean fungal growth on X, Y and Z were 1.5 x 10^3cfu/g ± 0.2x10^3 , 5.3x10^2 cfu/g ± 0.08x10^3 and 0.9 x 10^3 cfu/g ± 0.091 x10^3 at moisture contents 22.36±0.16, 23.61±0.67 and 23.13±0.52 respectively. Population growth pattern varies with the different treatments; it doubles every fourth week in X, it continues geometrically to stabilise between the eight and tenth week in Y and less gradual in Z. Microbial development was significantly different among various treatments (p< 0.05). Fungi associated with powdered neem leaves on fillets were *Aspergillus flavus, Penicillum citrium, A. parasiticus* and *A. niger* in order of importance.

Though neem was found to inhibit fungal growth on mustard seeds, growth seems not to have been inhibited on *C. nigrodigitatus*, however mycotoxin production ability could have been reduced. There is need to determine the mycotoxin production level of fungi associated with dried fish preserved with powdered neem leaves.

Key words: neem, microbes, smoked-dried fish

I. INTRODUCTION

Fish and fish products are important in development strategies of many developing countries especially the Least Developing Countries (LDCs), Small and Vulnerable Economies (SVEs) and Small Islands Developing States (SIDS). The fisheries sector is a large source of employment, a key dietary input and an important element of local livelihood [1]. One of the major courses of excessive pressure on fisheries resources is the need to make profit or at least to break even. Fishermen's response to low revenue has always been increased pressure which has contributed to the problem of over exploitation of many stocks in the world. Losses in fish production have been estimated at between 20 and50% due to poor handling and processing and preservation especially in the humid tropics [2]. Solving the problem of fish production requires a composite approach which should include better post harvest handling and preservation strategies.

Artisanal fishermen have various means by which they try to increase the shelf life of fish, these include smoke drying, sundrying, salt-sundrying and salted smoke drying. Studies have shown that these preservation methods have not been effective in extending the shelf life of fish and its products especially with respect to fungal invasion [3, 4]. While bacteria were identified as major spoilage organisms for fresh fish, dried fish are attacked by fungi [5]. Some of the fungi found to be associated with fish in the tropics include those of the *Aspergillus* sp., *Penicillum* sp., *Candida* sp. *Fusarium*. Neem products have been successfully used as antifungal agents in some plant food items[6].

This study investigated the efficacy of powdered neem as a preservative agent on the fillets of *Chrysichthys nigrodigitatus*; in search of alternatives to chemical antimicrobial agents in response to health concerns and economic reasons.

II. MATERIALS AND METHODS

Fillets of 50g ± 5 were prepared, treated with 30% brine and hot smoked to constant weight in an enclosed coal oven. Smoke was supplied from hard wood. Samples were divided into three parts; X was dusted with air dried powdered neem leaves, Y with freshly extracted vegetable oil, and Z was untreated. Products were packaged in airtight polythene

containers, stored and monitored for microbial development for 12 weeks. Nutrient quality was determined [7]. Data was analysed using SPSS 15 for windows.

III. RESULTS

The prevailing environmental conditions are present on Table 1. Mean values were temperature was 28^0C, relative humidity 60%, rainfall 58mm.

Table 1: Prevailing environmental conditions

Factor	Maximum	Mean	Minimum
Temperature (^{0}C)	31.35	28.0	25.50
Wind speed (Km/hr*)	3.1		
Relative humidity (%)	64	60	55
Rainfall (mm)*	65.4	58	30.7

**Source Ogun State, Nigeria, Ministry of Environment*.

The values of nutritional properties of the products were protein, 55.17 ±1.7, 54.75±1.04 and 55.15±1.73; moisture 22.36±2.57, 23.62±1.97 and 23.13±2.56 and lipids 5.87±0.21, 5.81 ±0.21 and 5.77±0.21 respectively (Table 2).

Table 2: mean values of nutrient composition of the different products (%)

	X	Y	Z
Protein	55.47 ±1.7	54.75±1.04	55.15±1.73
Lipid	5.87±0.21	5.81 ±0.21	5.77±0.21
Fibre	1.64±0.19	1.60±0.19	1.59±0.19
Ash	14.47±0.60	14.24±0.60	14.26±0.60
Moisture	22.46±2.57	23.62±1.97	23.13±2.56

A. Microbial population

Weekly mean population for the microbes and the moisture conditions are presented on Tables 3 and 4. Fungi growth was $5.12\ x10^2 \pm 0.22x10^2$, $1.30x10^2 \pm 0.22x10^2$ and $4.10x10^2 \pm 0.22x10^2$ for X, Y and Z respectively at the end of week 2 and at the end of the twelfth week it was $2.44x10^3 \pm 0.70x10^2 9.70x10^3 \pm 1.20x10^2 1.34x10^3 \pm 0.80x10^2$ (Table 3). Bacterial population at the end of week 2 was $5.12x10^2 \pm 0.22x102$, $1.30x10^2 \pm 0.22x10^2$ and $4.10x10^2 \pm 0.22x10^2$ while at the end of week 12 it was $2.44x10^3 \pm 0.70x10^2$, $9.70x10^3 \pm 1.20x10^2$ and $1.34x10^3 \pm 0.80x10^2$ for X, Y and Z respectively (Table 4).

Table 3: Bi-weekly mean population of fungi (cfu/g)

Treatment	Week	X	Moisture %
X	**2**	$5.12x10^2 \pm 0.22x10^2$	21.83±0.1
	4	$9.30x10^2 \pm 2.20x10^2$	21.69±0.6
	6	$1.14x10^3 \pm 1.10x10^2$	22.01±0.05
	8	$1.64x10^3 \pm 5.90x10^2$	22.94±0.07
	10	$2.32x10^3 \pm 1.50x10^2$	24.44±0.06
	12	$2.44x10^3 \pm 0.70x10^2$	26.99±0.02
Y	**2**	$1.30x10^2 \pm 0.22x10^2$	21.48±0.05
	4	$3.20x10^2 \pm 0.50\ x10^2$	21.43±0.07
	6	$6.20x10^2 \pm 0.13x10^2$	21.40±0.06
	8	$7.10x10^2 \pm 1.10x10^2$	22.06±0.65
	10	$8.35x10^2 \pm 1.30x10^2$	23.23±1.33
	12	$9.70x10^3 \pm 1.20x10^2$	23.53±2.04
Z	**2**	$4.10x10^2 \pm 0.22x10^2$	21.41±0.06
	4	$5.90x10^2 \pm 0.90x10^2$	21.41±0.09
	6	$8.60x10^2 \pm 0.22x10^2$	21.81±0.62
	8	$1.09x10^3 \pm 0.14x10^2$	22.73±1.33
	10	$1.14x10^3 \pm 0.40x10^2$	23.35±1.45
	12	$1.34x10^3 \pm 0.80x10^2$	23.55±0.05

Table 4: Mean weekly population of bacteria

Treatment	Week	X	Moisture
X	**2**	$1.86x10^3 \pm 0.17x10^2$	21.83±0.1
	4	$2.46x10^3 \pm 0.66x10^2$	21.69±0.6
	6	$2.92x10^3 \pm 0.66x10^2$	22.01±0.0 5
	8	$3.90x10^4 \pm 1.12x10^2$	22.23±0.0 7
	10	$4.84x10^4 \pm 0.66x10^2$	23.44±0.0 6
	12	$5.06x10^4 \pm s0.41x10^2$	23.55±0.0 2
	Mean total	$3.50x10^4 \pm 1.34 \times 10^{3a}$	22.36±0.1 6
Y	**2**	$1.50x10^3 \pm 0.22x10^2$	21.48±0.0 5
	4	$2.52x10^3 \pm 0.50 \times 10^3$	21.43±0.0 7
	6	$4.20x10^3 \pm 0.13x10^2$	21.40±0.0 6
	8	$6.10x10^3 \pm 1.10x10^2$	22.06±0.6 5
	10	$2.10x10^4 \pm 1.30x10^3$	23.23±1.3 3
	12	$4.70x10^4 \pm 1.20x10^3$	23.53±2.0 4
	Mean total	$1.73 \times 10^4 \pm 0.87x10^{3b}$	23.61±0.6 7
Z	**2**	$4.10x10^3 \pm 0.22x10^2$	21.41±0.0 6
	4	$5.90x10^3 \pm 0.90x10^2$	21.41±0.0 9
	6	$8.60 \times 10^3 \pm 0.22x10^2$	21.81±0.6 2
	8	$1.09x10^4 \pm 0.14x10^3$	22.73±1.3 3
	10	$3.14 \times 10^4 \pm 0.40x10^3$	23.35±1.4 5
	12	$4.34x10^4 \pm 0.80x10^3$	23.55±0.0 5
	Mean total	$2.27x\ 10^4 \pm 0.81 \times 10^{3c}$	23.13±0.5 2

Table 5. Mean population of fungi

Treatment	Population	Moisture
X	$1.5x\ 10^3 \pm 0.2 \times 10^{3a}$	22.36±0.16,
Y	$5.3x10^2 \pm 0.08x10^{3b}$	23.61±0.67
Z	$0.9x\ 10^3 \pm 0.09x10^{3c}$	23.13±0.52

** Values with different superscripts are significantly different*

Mean populations of fungi were significantly different from one another. Population was highest in X and least in Y (table 5). Similarly mean bacteria populations differ significantly from one another (Table 6)

Table 6. Mean population of bacteria

Treatment	Population	Moisture
X	$3.50x10^4 \pm 1.34 \times 10^{3a}$	22.36±0.16
Y	$1.73 \times 10^4 \pm 0.87x10^{3b}$	23.61±0.67
Z	$2.27x\ 10^4 \pm 0.81 \times 10^{3c}$	23.13±0.52

** Values with different superscripts are significantly different*

DISCUSSION

Estimated nutrient compositions are presented on Table 2. Protein and ash values estimated for the three products were higher than those estimated for smoked *C. nigrodigitatus* in previous studies [3], beef and pork [8] and were within the ranges recommended by US/RDA[9] .

Fungi found to be associated with smoked *C nigrodigitatus* in all the treatments were *Penicillum citrium, A. parasiticus, Aspergillus flavus,* and *A. niger*, similar fungi were identified in various smoked fish species displayed for sale in some West African markets[3,4]. Those associated with fillets treated with powdered neem leaves were *Aspergillus flavus, Penicillum citrium, A. parasiticus* and *A. niger* in the order of importance.

Population growth pattern varies with the different treatments; it doubles every fourth week in X, it continues geometrically to stabilise between the eight and tenth week in Y and less gradual in Z (Table 3). Bacterial population was more rapid in X and Z than in Y (Table 4).

Microbial development was significantly different among various treatments ($p< 0.05$). The mean population of microbes was higher in X than Y and Z (Tables 5 & 6).

Oil film possibly blocks the air passages thus impairing the respiratory activities that slows down the growth of microbes in Y. Microbial products in Z seem to have been better slowed down than those of X by the brine.

CONCLUSION

The effectiveness of neem in preventing fungi growth on smoked *C. nigrodigitatus* was not clearly detected from this study. However neem has been found to effectively slow down fungal growth in

some plants products. It was also proven to have effectively hinder the production of aflatoxins associated with *Aspergilus spp*. Though neem was found to inhibit fungal growth on mustard seeds [10], growth seems not to have been inhibited on *C. nigrodigitatus*, however mycotoxin production ability could have been reduced. There is need to determine the mycotoxin production level of fungi associated with dried fish preserved with powdered neem leaves.

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