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EFFECT OF *Moringa oleifera* MARINADE ON MICROBIAL STABILITY OF SMOKE-DRIED AFRICAN CATFISH (*Clarias gariepinus*)

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Abstract

The study examined the antimicrobial effect of Moringa oleifera marinade on smoke-dried catfish stored at ambient temperature (37±2^{0}C) for two months. The experimental treatments are the control, 1%, 2% and 3% (w/v) Moringa oleifera Marinade (MOM) and 5% Brine (w/v) solutions. Seventy-five fishes of average weight of 260±8g were gutted, washed and randomly assigned to the treatments. Thereafter, the fishes were soaked in the treatments for 2 hours and later hot smoked for 12hours. After smoking, the fishes were stored in netted boxes and placed on laboratory shelves for two months. Microbial counts were conducted at 7-day interval while biochemical tests were conducted on the 8th week. Seven bacterial species namely; Staphylococcus sp, Bacillus sp, Klebsiella sp, Corynebacterium sp, Pseudomonas sp, Escherichia coli and streptococcus sp and six fungal species namely; Penicillium italicum, Cladosporium sp, Neurospora crassa, Candida sp, Aspergillus niger and Saccharomyces cerevisiae were observed in the study. There was a general increase in microbial load as storage progressed. However, the increment was pronounced in the control and brine treated fish samples. In all levels of MOM and 5% Brine, there was decrease in the bacterial and fungal counts as compared with the control samples. 3% MOM exhibited the highest antibacterial potency while 5% Brine exhibited the highest antifungal potency. Moringa oleifera marinade could be use to protect stored smoke-dried catfish from microbial spoilage thus limiting economic loss and possible heath risk to consumers.

Key words: *Moringa, marinade, bacteria, fungi, catfish, smoke-dried*

Introduction

Fish is an indispensible source of animal protein, essential fatty acids, minerals and vitamins. Its amino acids composition very well suited human dietary requirements, competing favorably with egg, milk and meat in its nutritional value (Feldhusen, 2000). Fish protein is relatively cheaper and richer in lysine and other sulphur amino acids than other livestock protein thus suitable for complementing high carbohydrate diets (Abdullahi *et al*., 2001). Fish is highly perishable. It is readily susceptible to chemical and microbial deterioration leading to economic loss, reduction in quality attributes and wastage (Gram and Huss, 2001). Food wastage and spoilage has been recognized as a significant constraint in achieving the much-desired self-sufficiency in food and fibre production in Africa (FAO, 2000). Presently, about two-third of the world population subsist on poorly balanced diets that retard normal growth and development (FAO, 2012). Maintenance of high quality fish therefore call for adequate, effective and affordable preservative techniques to enhance preservation of this protein resources. Post harvest losses of fish may reach 35%; in some cases are nearly 25million tones of the world's catch and in some developing countries, post harvest losses of fish exceed those of any other commodity, often surpassing 50% of the landed catch (FAO, 2000).

An estimated 40% of total fish landing in Nigeria is lost as post harvest losses (Eyo, 2001). It was estimated that 20 to 50% of the fish produced in the remote costal centers and many tropical countries perish before they reach consumers due to the poor handling, preservation and processing practices adopted by artisanal fishermen, fish farmers and fisheries entrepreneurs (Eyo, 2001). In addition, significant quality is lost through the absence of adequate technology and expertise to prevent losses in many tropical countries (Clucas, 1990). In order to curb fish spoilage, increase shelf life and add value to products, various preservation techniques are

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employed. These include chilling, freezing, salting, canning, drying and smoking (Kumolu *et al*., 2010). However, smoking is the most popular method of fish processing in Nigeria (Bako, 2005).

Fish smoking is particularly relevant in the artisanal fisheries sector in that it prolongs the shelf life of the fish, enhances flavor and increases utilization of the fish in addition to reducing waste as well as increasing protein availability to people (Jallow, 1995). However, smoke-dried fish are liable to microbial damage leading to loss of valuable nutrients and reduced shelf life. Microbial spoilage could predispose consumers to health hazards resulting from food poisoning (Gram *et al*., 2000). The objective of this study was to evaluate the effect *Moringa oleifera* marinade on microbial stability of smoked dried African catfish stored for two months.

Material and Methods

Preparation of Marinade

Fresh moringa leaves were obtained from the University of Ilorin, Ilorin, Nigeria. The leaves were air dried at ambient temperature (37 ± 2^0C) for 4 days and ground into powder using food blender (Starlite, Model No: SL-999 CHINA). *Moringa oleifera* marinade (MOM) was prepared by adding separately specific quantity (10g, 20g or 30g) of *Moringa oleifera* leaf powder to 1000ml of water to form 1, 2 or 3% MOM respectively. 50g of salt was added separately to 1000ml of water to form 5% Brine solution. No additive was added to the control treatment.

Fish preparation

Seventy-five catfish samples were purchased from Osagbemi Farms in Ilorin metropolis. The catfishes are a monophyletic group, belonging to the super-order called the *Ostariophysi.* The catfish has a scale-less slimy skin, which is dark and pigmented in the dorsal and lateral parts of the body (Kiin-Kabari, *et al*., 2011).

The processing and smoking of the catfishes were carried out at Godbet Homestead Fish Farm, Basin road, Ilorin, Nigeria. The average weight of the fishes was 270±8g while the total weight was 20kg. The fishes were gutted using a sharp knife by cutting laterally from the end of the gill cover through the belly portion to the anus. Thereafter, they were thoroughly washed and rinsed. The total weight of the fish after gutting was 18.5kg with average weight of 250±6g.

Treatments

The fishes were randomly assigned to five experimental treatments. These are the Control, 1%, 2% and 3% MOM and 5% Salt (w/v) solution. Each treatment was replicated thrice with 5fishes/replicate. The fishes were soaked in the marinade for 2 hours. Thereafter, the fishes were set in the smoking kiln consisting of five-twin tiers and subjected to hot smoking for 12hours with charcoal as heat source. The tiers were interchanged every 3 hours to ensure uniform heat distribution and drying. The smoke-dried fishes were stored in air-free netted boxes to prevent flies contamination and to enhance flow-through ventilation throughout the storage period. The boxes were placed on laboratory shelves at room temperature (37 ± 2^0C) for 8 weeks.

Microbiological Analysis

Culture and Isolation of Microorganisms

The bacterial count was determined using Nutrient Agar (Lab M) while the fungi were determined using Potato Dextrose Agar (Lab M) to which 30µg/ml of streptomycin was added. Staphylococcal counts were determined using Mannitol Salt Agar (Biotec) while Coliforms were enumerated using MacConkey Agar (Biotec). All media were prepared according to manufacturer's instructions and sterilized at 121^oC, 15psi for 15 minutes. The methods of Fawole and Oso (2002) were used to culture, isolate and identify the organisms. The organisms were identified microscopically and based on their reactions to various biochemical tests.

Statistical Analysis

The microbial counts followed a 5x8 factorial arrangement in a completely randomized design. The data obtained were analyzed using analysis of the variance (ANOVA) model suitable for the design with the aid of Genstat discovery program package (fourth edition). The ANOVA model is as follow

$Y_{ij}= \mu+\alpha_{i+}\beta_j+(\alpha i \times \beta j) + e_{ij}$.

where Y_{ij} denotes the ij^{th} observation arising from level i of additive and level j of storage time.

μ=overall mean

α_i= effect of level i of additive

$\beta_{j=}$ effect of level j of storage time

$(\alpha i \times \beta j)$= interaction effect of additive and storage time

$e_{ij=}$ random error term

Results and Discussion

Table 1 Effect of additive and storage weeks on microbial load (cfu/g) of smoked dried catfish

Additive	Microorganism	Storage week							SEM
		1	2	3	4	5	6	7	8
Control		$1.0x10^{6}$	$4.3x10^{8d}$	$5.2x10^{7c}$	$>3.0x10^{8c}$	$4.0x10^{8b}$	$5.4x10^{7}$	$>3.0x10^{8}$	$>3.0x10^{8c}$
1%MOM	Bacteria	$1.0x10^{6}$	$2.4x10^{6a}$	$5.0x10^{7c}$	$6.3x10^{7a}$	$4.0x10^{8}$	$3.8\ x10^{7b}$	$8.9x10^{7}$	$5.0\ x10^{7b}$
2%MOM		$1.0x10^{6}$	$1.2x10^{7b}$	$1.6x10^{7b}$	$1.1x10^{8b}$	$3.0x10^{8a}$	$3.0\ x10^{8}$	$1.2\ x10^{8}$	$6.0\ x10^{7b}$
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3%MOM		$1.0x10^{6}$	$1.3x10^{7b}$	$1.0x10^{7a}$	$1.2\ x10^{8b}$	$3.0x10^{8a}$	$2.2\ x10^{7a}$	$8.0\ x10^{6}$	$7.0\ x10^{6a}$
5%Brine		$1.0x10^{6}$	$4.2x10^{7c}$	$1.0x10^{7a}$	$>3.0x10^{8c}$	$4.0x10^{8b}$	$4.0x10^{8}$	$3.0\ x10^{7}$	$2.0\ x10^{8c}$
		NS	**	**	**	**	**	**	**
Control		$9.0x10^{6b}$	$4.9x10^{7c}$	$5.0x10^{7c}$	$2.7\ x10^{7d}$	$3.0x10^{6c}$	$3.4x10^{7e}$	$3.9\ x10^{8d}$	$4.0x10^{8d}$
1%MOM		$4.5x10^{6c}$	$2.7x10^{7b}$	$2.8x10^{7b}$	$4.2\ x10^{6c}$	$2.8x10^{6c}$	$4.7x10^{6c}$	$5.00x10^{7c}$	$5.0x10^{7c}$
2%MOM	Fungi	$3.2x10^{6b}$	$2.2x10^{7b}$	$5.0x10^{7c}$	$4.0\ x10^{6c}$	$3.0x10^{6c}$	$9.0x10^{6d}$	$1.6x10^{7a}$	$2.0x10^{7a}$
1340223									
3%MOM		$1.0x10^{6a}$	$2.0x10^{6a}$	$5.0x10^{7c}$	$3.0x\ x10^{6b}$	$2.5x10^{6a}$	$2.0x10^{6a}$	$7.0x10^{6b}$	$4.0x10^{7b}$
5%Brine		$1.0x10^{6a}$	$3.0x10^{6a}$	$1.2x10^{7a}$	$1.6\ x10^{6a}$	$2.0x10^{6a}$	$4.0x10^{6b}$	$1.1\ x10^{7a}$	$2.0x10^{7a}$
		**	**	**	**	**	**	**	**

a, b, c, d, e means having different superscript along the same column are significantly different ($P<0.01$). NS=Non-significant. **=Significant. MOM=*Moringa oleifera* marinade

Table 2 Bacterial and fungi isolates obtained from smoke-dried fish samples

Treatment	Bacterial Isolate	Fungi Isolate
Control	*Corynebacterium sp, Streptococcus sp,Cladosporium sp, Pseudomonas sp, Staphylococcus aureus, Bacillus sp*, *Escherichia coli*	*Neurospora crassa, Rhizopus nigricans, Candida sp, Aspergillus niger, Saccharomyces cerevisiae*
1% MOM	*Streptococcus sp, Micrococcus sp, Staphylococcus sp, Staphylococcus aureus, Pseudomonas sp, Bacillus sp*	*Neurospora crassa, Mucor sp, Saccharomyces cerevisiae*
2% MOM	*Staphylococcus aureus, Bacillus sp*	*Mucor sp, Rhizopus nigricans , Aspergillus niger*
3% MOM	*Klebsiella sp, Staphylococcus aureus, Bacillus sp*	*Penicillium italicum*
5% Brine	*Corynebacterium sp, Pseudomonas sp, Bacillus sp*	*Penicillium species II, Rhizopus nigricans, Aspergillus niger*

There were significant differences in the microbial load of smoked-dried fish samples treated with various additives on different storage weeks (Table 1). In all levels of MOM, there was decrease in the bacterial and fungal loads (Table 1) as compared with the untreated fish samples (Control). Different parts of *Moringa oleifera* plant contain different phenolics (Fahey, 2005) and the rare combination of zeatin, quercetin, kaempferom among many other phytochemical compounds (Bukar *et al*., 2010). These compounds might be responsible for the significant decrease in the amount and variety of microorganisms isolated in the Moringa treated samples as opposed the control and brine-treated samples. Fish is a low-acid food (Haruna, 2003) that can readily support the growth of pathogens, particularly bacteria if not properly handled and rapidly processed after harvesting. This partly explains why despite the fact that all the fish samples showed growth of heterotrophic bacteria and fungi throughout the study, the bacterial load was consistently higher than the fungal load. The bacterial population of the samples ranged from 1.00×10^6 - 4.00×10^8 cfu/g (Table 1). The least figure of 1.00×10^6 was observed in all samples 24 hours after smoking (week 1). The homogeneity in bacteria load observed in the first week of the study could have resulted from the fact that the fish samples were analyzed for microbial load just a day after removal from the smoking kiln. The highest bacterial load of 4.3×10^8cfu/g was found in the control samples at the end of the first week. However, for all samples there was an initial rise in bacterial load and then a general decline in the bacterial population by the third week, whereas, there was an increase in fungal counts (cfu/g) in all samples over the entire period of storage. This could be due to the decreasing moisture content with time, which made the fish less favorable for the growth of bacteria (Gbolagade *et al*., 2012) but encouraged fungal growth. The proliferation of the fungi could also result in the production of various antibacterial, compounds that could retard or inhibit the growth of some bacteria and thus eliminate competition. For instance, *Penicillium sp* produces the broad-spectrum antibiotic penicillin, which could be responsible for inhibiting the bacteria over time. There was a general increase in bacterial load as storage week progresses. However, the increment was more pronounced in control and brine treated samples. All levels of *Moringa oleifera* marinade (MOM) incurred a significant antibacterial effect that was concentration dependent with 3% MOM having the lowest bacteria load. This observation corroborates the findings of Napoleon *et al*., (2009) who reported that Moringa leaf ethanol extract had a broad, spectrum activity on food borne bacteria isolate. Its' activity is mainly focused on four bacterial isolate which are *Ecterobacter sp, S. aurens, P.aeruginosa and E. coli*. The antimicrobial activity of moringa marinade could be due to the presence of some phytochemicals, but most importantly due to the activity of a short polypeptide named 4 (a-L-rhamnosyloxy) benzyl-isothiocyanate (Guevara *et al*., 1999). Studies have shown that moringa chloroform and ethanol extracts are potential sanitizers and or preservatives, this is because they were found to possess antimicrobial activities against some food borne microorganisms often implicated in spoilage of foods and food borne illness (Bukar *et al.,* 2010).

The bacteria identified (Table 2) comprised mainly of normal flora of fishponds and skin of fish processors (Fafioye, 2011) and these included members of the genera *Escherichia, Bacillus, Pseudomonas* and *Staphylococcus.* The most commonly isolated bacterial species was *Staphylococcus aureus,* which is a normal flora of many water bodies (Le Chevallier and Seidler, 1980) and the skin (Davis, 1996; Todar, 2012) of the fish handlers. This finding is in agreement with that of Ehizibolo *et al*., (2007) who reported a 44.6% prevalence rate for *Staphylococcus aureus* in smoked catfish and tilapia sold in Jos, Nigeria. However, the counts for *S. aureus* were lower than the benchmark values (for meat) of 100cfu/g (Ehizibolo *et al*., 2007). *Escherichia coli* was only isolated in the untreated sample thus indicating that the treatment of fish with *Moringa oleifera* extracts is useful in the elimination of *E. coli*. Salihu *et al*., (2008) gave similar report of the occurrence of *E. coli* and *Pseudomonas sp* in smoked fish. *E. coli* and *Pseudomonas sp* are pathogens that can cause intestinal infections and nosocomial infections respectively in humans (Todar, 2012). It is however noteworthy that contrary to the reports of Ehizibolo *et al*., (2007) and Salihu *et al.,* (2008), the common human pathogen *Listeria monocytogenes* was not detected

in the fish samples. Another important pathogen, *Salmonella sp* was not found in any moringa treated fish. This observation is in line with the report of Nwaiwu *et al*., (2011) who showed activity of Moringa oleifera hexane extract against *Salmonella, Shigella* and *Enterobacter*. However, this observation was contrary to the findings of Onyia, et al; (2011) in which one of the bacteria isolated from smoked fish was *Salmonella sp*. The discrepancy in the present study and that of Onyia *et al*., 2011) may be attributed to proper aseptic techniques maintained at every stage of the experiments and the action of the *Moringa oleifera* marinade. This result is corroborated by the report of Bukar *et al.* (2010) that ethanol extract of moringa displayed bioactivity on pathogenic bacteria such as *E. coli, S. aureus, P. aeruginosa, S. typhimurium* and *Enterobacter aerogenes.* 3% MOM was the most effective in suppressing the bacterial population while 5% Brine was most effective at keeping down fungal counts.

The fungi recovered in the fish samples (Table 2) have been reported to be regular contaminants of smoked fish (Abolagba *et al.,* 2011; Wogu and Iyayi, 2011). It was impressive that *Aspergillus flavus* and *Fusarium* spp among other toxigenic fungi were not encountered in the samples. *Neurospora crassa* and *Saccharomyces cerevisiae* have also been reported in smoked oysters (Odu *et al.,* 2012). The occurrence of *Escherichia coli* in control samples is suggestive of faecal contamination of the water from which the fishes were reared because *E. coli* is an indicator organism and its presence in water or water products indicates the likely presence of feces and by extension, the presence of other pathogenic intestinal microorganisms (Edberg *et al*., 2000).

Conclusion and Recommendation

All levels of *Moringa oleifera* marinade caused significant reduction in microbial load of smoke-dried catfish. There was a general increase in microbial counts as storage progressed. However, the increment was pronounced in control and brine treated samples. 3% MOM exhibited the highest antibacterial effect while 5% Brine exhibited the highest antifungal potency. This was closely followed by all *Moringa oleifera* samples. The control samples had diverse microflora than other treatments. The 3% MOM treated samples showed the least bacteria growth.

It is thus recommended that this preparation be incorporated into fish before smoking for additional preservative effect. *Moringa oleifera* marinade could be used to stabilize the microbial load of smoke-dried catfish stored at ambient temperature for up to two months. This will go a long way in reducing fish spoilage especially due to bacteria and protect consumers against food borne diseases thus enhancing food safety and security. Further studies should be carried out to determine the synergistic effect of brine and *Moringa oleifera* marinade on microbial stability of smoke-dried catfish stored for longer period under ambient temperature.

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