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Assessment of Moringa Oleofera Seed Powder on the Shelf Life of Smoked Clarias Gariepinus

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Abstract

Assessment of moringa oleofera seed powder on the shelf life of smoked Clarias gariepinus. Fifteen table sized C. gariepinus with an average weight of 550g were used and were assigned into five treatments. The five treatments were soaked in M. oleifera marinade (MOM) at different inclusion level. These are: the control with 0% MOM, 10g MOM inclusion, 20g MOM inclusion, 30g MOM inclusion and 40g MOM inclusion in 1000cm3 of water. The fish were gutted, washed and randomly assigned to the treatments. Thereafter, the fishes were soaked in the treatments for one hour and later hot smoked. After smoking, the fishes were stored in netted boxes and placed on laboratory shelves for two months. Microbial counts and proximate composition were conducted on the various treatments at 7-day interval for four weeks. There was a general increase in microbial load as storage progressed. There was a general increase in microbial load as storage progressed. However, the increment was pronounced in the control fish samples. In all levels of MOM, there was decrease in the bacterial and mold/yeast counts as compared with the control samples. 4% MOM exhibited the highest antibacterial potency and antifungal potency as well. Of all the nutrients which are Protein, fat, fibre, ash, moisture and carbohydrate, only protein and moisture increased generally as storage weeks progressed. The other nutrients decreased. M. oleifera marinade could be used to protect stored smoked catfish from microbial spoilage thus limiting economic loss and possible health risk to consumers. It was concluded that using M. oleifera seed as a preservative for smoked C. gariepinus enhances the nutrient quality and reduces the microbial loads. Therefore, preservation of feed with M. Oleifera seed should be encouraged.

Introduction

Fish is an indispensable 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 (Food and Agriculture Organization, 2000).

Presently, about two-third of the world population subsist on poorly balanced diets that retard normal growth and development (Food and Agriculture Organization, 2012). Shelf life is defined as the period of time a product is fit for consumption; it is a relatively short period for fresh fish stored under refrigerated conditions (12

days). The limit of the shelf-life which can be determined based on sensory, chemical and microbial criteria is affected by the rate of enzymatic reactions and the number and species of microorganisms affecting the products storability. Other determining factor is the handling temperatures which must be evaluated throughout the processing stages (Chowdburg et al., 2007).

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 (Food and Agricultural Organization, 2000). An estimated 40% of total fish landing in Nigeria is lost as post-harvest losses. 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 order to curb fish spoilage, increase shelf life and add value to products, various preservation techniques are 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). Smoking is the process through which volatiles from thermal combustion of wood penetrate meat or fish flesh (Simko, 1991). 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).

M. oleifera (commonly called drumstick) is one of such most important terrestrial plants which exhibit antibacterial, antifungal as well as antioxidant properties which influence its application in food preservation. Due to the presence of many important substances like ascorbic acid, estrogenic substances, beta sitosterol, iron, calcium, phosphorus, copper, vitamin A, B and C, alpha tocopherol, beta carotene, protein and essential amino acids like methionine, cystine, tryptophan and lysine, drumstick leaves can be considered as a dietary supplement. Ethanolic extract of M. oleifera showed a broad spectrum antimicrobial property against many pathogens including Staphylococcus aureus, Escherichia coli, Bacillus sp., Pseudomonas aeruginosa, Cornebacterium sp., Klebsiella pneumonia and Acinetobacter sp. (Rajamanickam and Sudha, 2013) due to presence of phytochemicals such as flavonoids, saponins, tannins and other phenolic compounds (Sato et al., 2004). Fish 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). To satisfy consumers' demand, it is necessary to produce good-quality and safe smoked fish products. Maintenance of high quality fish therefore calls for adequate, effective and affordable preservative techniques and preservatives to enhance preservation of this protein resource. The aim of this study is to assess moringa oleofera seed powder on the shelf life of smoked *C. gariepinus*. The objective is to determine the level of microbial loads and the nutrient quality of smoked *C. gariepinus* preserved with *M. oleifera* seed marinade.

Materials and Methods

Study Area

The experiment was carried out at the Department of Fisheries and Aquaculture, Adamawa State University, Mubi, Nigeria. Mubi is located in the Northeastern part of Adamawa State in Nigeria. It lies on latitude 10°32'N to 10°11'N and longitude 13°12'E to 13°35'E, with a total land mass of 506.4Km^2 and a population size of 759,045 people. (Adebayo, 2004)

Collection and Preparation of *M. oleifera* Seed

Dried seeds of M. oleifera were purchased from Michika Market and were brought to the laboratory and were grounded into powder for the experiment. The M. oleifera seeds was peeled and grounded into powder using an electric blender. M. oleifera seed marinade (MOM) was prepared by adding separately specific quantity (10g, 20g, 30g and 40g) of M. oleifera seed powder to 1000ml of water to form 1%, 2%, 3% and 4% MOM respectively. 50g of salt was added separately to the1%, 2%, 3% and 4% MOM. Only 50g of salt was added separately to 1000ml of water to form 5% Brine solution to serve as control treatment. (Adeyemi et al., 2013)

Collection and Preparation of Fish Sample

Fifteen life C. gariepinus fish species with an average weight of 550g were purchased from the School Farm of the Department of Fisheries and Aquaculture, Adamawa State University, Mubi. The samples were brought to the smoking unit of the Department. The fish were

killed by brining and were washed properly to remove the slime on them. They 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 rinsed. (Adeyemi et al., 2013)

Treatments

The fish were randomly assigned to five experimental treatments. These are the Control (having 5% salt solution), 1% MOM, 2% MOM, 3% MOM and 4% MOM. Each treatment was replicated three times. The fish were soaked in the marinade for 1 hour. Thereafter, the fish were removed from the marinade and were drained properly. (Adeyemi et al., 2013)

Fish Smoking and Storage

Fire was set in the smoking kiln with charcoal as heat source, until the trays in the kiln were heated enough that hands cannot be laid upon for 5 seconds. The fish was set in a charcoal smoking kiln subjected to hot smoking until a constant weight was achieved. The smoke from the kiln was produced by the burning of charcoal. Uniform heat distribution and drying were ensured by exchanging the trays. This was followed by sun-drying to make fish muscle compressed and facilitate to prevent breaking of smoked products. The smoked fish 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 for four weeks. (Adeyemi et al., 2013)

Microbial Analysis

All microbial analysis was done following the methods prescribed by (AOAC, 2000) for four weeks in which the same analysis was conducted in the initial week.

Total Bacterial count

Total bacterial count was done using pour plate method of (AOAC 2000). It was calculated and expressed as colony-forming units per gram (cfu/g).

Mold and yeast counts

Mold and yeast counts were done by plating out serially diluted samples on yeast and mold agar. These were calculated and expressed as colony-forming units per gram (cfu/g).

Proximate Composition Analysis

The smoked catfish samples were finely ground and homogenized for proximate analysis. The percentage proximate composition of the smoked fish samples was determined according to the AOAC (2010) methods. The percentage proximate composition was analyzed for four weeks as it was also done in the initial week.

Determination of protein

The total protein content was estimated using the Kjeldahl method which includes digestion, distillation and titration as recommended by AOAC (2010). The percentage protein was obtained using the formula:

$$\% \text{ N} = \frac{0.00014\ X\ 1\ litre\ X\ 50\ X\ 100}{wt.of\ feed\ stuff\ taken} \times 100$$

% protein = N X 6.25

Determination of fat

Fat was determined by sox let method as recommended by Association of official analysis AOAC (2010). The percentage fat was obtained.

The formula: $\%\text{fat} = \frac{W1-W0}{weight\ of\ sample}$ x 100

Where W_0 = initial weight of fat

W_1 = final weight of fat

Determination of fibre

Fibre was determined using trichloroacetic acid method as recommended by AOAC (2010). The percentage of the crude fibre was obtained using formula:

% fibre = $(W_1 - W_0)$ x 100
Where W_0 = initial weight
W_1 = final weight

Determination of ash

Ash weight was analyzed using oven drying method as recommended by AOAC (2010). The percentage of ash was obtained using the formula:

$$\%\ Ash = \frac{wt\ \ of\ dish + ash - wt\ \ of\ dish}{wt.of\ feed\ stuff\ used} \times 100$$

Determination of moisture

The moisture content was determined by oven drying samples overnight at 105°C until constant weight. Percentage moisture was calculated using formula:

$$\% \text{ Moisture} = \frac{(\text{wt of dish} + \text{feed stuff before drying}) - \text{wt of dish} + \text{feed stuff after drying})}{\text{wt. of feed stuff taken}} \times 100$$

Determination of Carbohydrate

Carbohydrate was determined using differential method as recommended by AOAC (2010). The percentage of carbohydrate was obtained using the formula:

% CHO = 100 – (% moisture + % Ash + % fibre + % protein + % crude fibre)

Data Analysis

Data obtained in this study were analyzed using analysis of variance (ANOVA) procedures (SPSS 11.0 for Windows). Differences between the mean values of the treatments was determined by the least significant difference (LSD) test and the significance was defined at $P<0.05$.

Results

Determination of microbial loads of smoked *C. gareipinus* preserved with *M. oleifera* seed marinade

The result for microbial analysis is presented on Table 1. The result shows that the highest level of bacterial load was 7.5 x 10^6cfu/g obtained in week 4 of 1% MOM treated fish sample. The least value of the bacterial load was8.9 x 10^3cfu/g and was recorded in week 4 of the 4% MOM treated fish sample. For the mold and yeast counts, the highest value (6.7 x 10^5cfu/g) was recorded in week 4 of 1% MOM treated sample, and the least value (7.7 x 10^2cfu/g) was recorded in week 1 of 4% MOM treated sample. There was an increase in bacterial load as storage week progressed from week 1 to week 4 in 1% MOM treated sample. In 2% treated sample, bacterial load increased from week 1 to week 3 but decreased in week 4 a little. There was a general control of bacterial load in 3% MOM and 4% MOM treated sample. However, bacterial load control was more pronounced in 4% MOM treated sample. Therefore, only 3% and 4% levels of MOM incurred a significant antibacterial effect. This observation agrees with the findings of Rajamanickam and Sudha, (2013), who reported that M. oliefera exhibit antibacterial, antifungal as well as antioxidant properties which influence its application in food preservation due to the presence of many important substances like ascorbic acid.

The mold and the yeast had a general increase with the storage weeks. However, the 4% MOM treated sample exhibited much control over the mold and yeast. A wide range of different foods can be spoiled by mold and yeast (Stephanie, 2014). 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).

Microorganisms	Treatments	Initial	Storage		Weeks	
			1	2	3	4
Total Bacterial Count (cfu/g)	T1	6.3 x 10^4	8.5 x 10^5	8.9 x 10^5	7.9x 10^6	8.5 x 10^6
	T2	4.5 x 10^3	8.2 x 10^5	8.5 x 10^5	7.5 x 10^6	6.1 x 10^6
	T3	3.3 x 10^3	8.9 x 10^4	7.3 x 10^5	7.3 x 10^5	4.4 x 10^5
	T4	3.2 x 10^3	8.7 x 10^4	8.6 x 10^4	7.7 x 10^4	5.8 x 10^4
	T5	3.2 x 10^3	6.5 x 10^4	6.3 x 10^4	3.1 x 10^4	8.9 x 10^3
Moulds and Yeast (cfu/g)	T1	4.5 x 10^3	7.8 x 10^3	8.4 x 10^4	6.2 x 10^5	8.1 x 10^5
	T2	1.4 x 10^3	5.7 x 10^3	5.4 x 10^4	4.8 x 10^5	6.7 x 10^5
	T3	3.7 x 10^3	3.3 x 10^3	4.8 x 10^4	8.7 x 10^4	2.4 x 10^5
	T4	1.8 x 10^3	2.8 x 10^3	6.1 x 10^3	7.1 x 10^3	1.8 x 10^4
	T5	8.5 x 10^2	7.7 x 10^2	3.7 x 10^3	5.2 x 10^3	8.1 x 10^3

T%=Control (0% MOM), T1=1% MOM T2=2% MOM T3=3% MOM T4=4% MOM

Table 1: *Microbial loads of smoked C. gareipinus preserved with M. oleifera seed marinade.*

Determination of nutrient quality of smoked *C. gareipinus* preserved with *M. oleifera* seed marinade

The result for the nutrient quality is presented on Table 2. The result shows that protein had the highest value (60.43%) in week 4 of 4% MOM treated sample and the least value (51.95%) in week 1 of 1% treated sample. Fat had the highest value in week 1 of 1% treated sample and the least in week 3 of 4% treated sample. Fish sample with 1% MOM in week 1 showed the highest content (0.69%)

of fibre and the sample with 3% and 4% MOM had the least value (0.70%) in week 4. The ash content had the highest value (7.15%) in week 1 of 1%, 2% and 3% MOM while the least value (6.85%) in week 3 of 2%, 3% and 4% MOM. Moisture content was highest (4.00%) in week 4 of all the treatments (1%, 2%, 3% and 4%) and the least (3.00) in week 1of 1% MOM treated sample. Carbohydrate content had the highest value (20.73%) in week 1 of 1% treated sample and had the least value (13.91%) in week 4 of 4% treated sample.

Proximate Composition	Treatments	Initial	Storage		Week	
			1	2	3	4
Protein %	T0	50.26^{e}	51.21^{e}	53.00^{e}	55.05^{e}	55.71^{e}
	T1	52.16^{d}	51.95^{d}	54.05^{d}	56.19^{d}	56.90^{d}
	T2	53.21^{c}	54.78^{c}	55.71^{c}	57.12^{c}	58.06^{c}
	T3	53.63^{b}	54.93^{b}	56.34^{b}	57.61^{b}	58.66^{b}
	T4	56.27^{a}	56.81^{a}	56.95^{a}	58.70^{a}	60.43^{a}
Fat %	T0	17.96^{a}	16.46^{a}	16.12^{a}	15.91^{a}	14.96^{a}
	T1	16.86^{b}	16.21^{b}	16.00^{b}	15.43^{b}	14.28^{b}
	T2	16.22^{c}	15.00^{c}	15.34^{c}	15.00^{c}	14.03^{c}
	T3	15.96^{d}	14.78^{d}	14.16^{d}	13.76^{d}	13.55^{d}
	T4	15.33^{e}	14.03^{e}	14.00^{e}	13.00^{e}	13.22^{e}
Fibre %	T0	1.10^{d}	0.95^{b}	0.90^{a}	0.85^{b}	0.75^{a}
	T1	1.15^{c}	0.96^{a}	0.90^{a}	0.85^{b}	0.72^{b}
	T2	1.16^{c}	0.93^{c}	0.91^{a}	0.92^{a}	0.72^{b}
	T3	1.23^{b}	0.87^{d}	0.85^{b}	0.85^{b}	0.70^{c}
	T4	1.27^{a}	0.85^{e}	0.83^{c}	0.79^{c}	0.70^{c}
Ash %	T0	7.00^{a}	7.25^{a}	7.36^{a}	7.05^{a}	6.90^{a}
	T1	6.85^{b}	7.15^{b}	7.30^{b}	7.00^{b}	6.70^{b}
	T2	6.80^{c}	7.15^{b}	7.20^{c}	6.85^{c}	6.70^{b}
	T3	6.80^{c}	7.15^{b}	7.20^{c}	6.85^{c}	6.70^{b}
	T4	6.53^{d}	7.00^{c}	7.20^{c}	6.85^{c}	6.70^{b}
Moisture %	T0	2.96^{e}	3.00^{c}	3.20^{c}	3.81^{c}	3.80^{b}
	T1	3.00^{d}	3.00^{c}	3.45^{b}	3.90^{b}	4.00^{a}
	T2	3.16^{c}	3.20^{b}	3.45^{b}	3.90^{b}	4.00^{a}
	T3	3.20^{b}	3.20^{b}	3.45^{b}	3.90^{b}	4.00^{a}
	T4	3.35^{a}	3.42^{a}	3.50^{a}	3.95^{a}	4.00^{a}
Carbohydrate %	T0	18.73^{d}	21.14^{a}	19.43^{a}	17.63^{a}	17.86^{a}
	T1	19.98^{a}	20.73^{b}	18.30^{b}	17.42^{b}	17.32^{b}
	T2	19.46^{b}	18.95^{c}	17.39^{c}	16.85^{c}	15.65^{c}
	T3	19.20^{c}	19.27^{d}	18.01^{d}	16.36^{d}	15.26^{d}
	T4	17.26^{e}	17.97^{e}	17.52^{e}	15.43^{e}	13.91^{e}

Means with the same superscript on the same row do not differ significantly (P<0.005)

Table 2: *Proximate composition of smoked C. gareipinus preserved with M. oleifera seed marinade.*

Discussion

There was an increase in bacterial load as storage week progressed from week 1 to week 4 in 1% MOM treated sample. In 2% treated sample, bacterial load increased from week 1 to week 3 but decreased in week 4 a little. There was a general control of bacterial load in 3% MOM and 4% MOM treated sample. However, bacterial load control was more pronounced in 4% MOM treated sample. Therefore, only 3% and 4% levels of MOM incurred a significant antibacterial effect. This observation agrees with the findings of Rajamanickam and Sudha, (2013), who reported that M. oliefera exhibit antibacterial, antifungal as well as antioxidant properties which influence its application in food preservation due to the presence of many important substances like ascorbic acid. The mold and the yeast had a general increase with the storage weeks. However, the 4% MOM treated sample exhibited much control over the mold and yeast. A wide range of different foods can be spoiled by mold and yeast (Stephanie, 2014). 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).On the whole, there were significant differences in proximate composition of the smoked fish samples on different storage weeks. Protein increased with the storage weeks. Protein content was generally high in all the weeks compared to other nutrients. This is so because fish is a good source of protein as reported by Tidwell, (2011). This was so, because M. oleifera seed as other legumes, are good source of proteins, as reported by Compaoré et al., (2011). Fat contents decreased generally through the storage weeks. The fat content reduced with increase in the percentage of MOM. Therefore 1% MOM treated sample had the highest fat value. This was in disagreement with the findings of Compaoré et al., (2011), who reported that M. oleifera seed contains fat as other legumes. The fibre contents also decreased along the storage weeks

with more pronouncement in the highest percentage (4% MOM) of the treated sample. This may be so because M. oleifera seed is a legume and has little fibre content in it (Compaoré et al., 2011). However, the general decrease in fibre content in this research agrees with the report of Ibrahim, (2017), who reported that fat content reduced after some months in fish samples. There was a decrease of ash contents across the storage weeks as reported by Ibrahim, (2017). However, more level of MOM recorded lower values of the ash contents. Moisture content increased with more level of MOM from week 1 to week 4 of the storage weeks. This disagrees with the report of Ibrahim, (2017), and could have been that M. oleifera seed absorbs moisture from the atmosphere. There was no significant difference of carbohydrates contents among the treated samples in the storage weeks. There was a general decrease in the carbohydrate contents from week 1 to week 4. M. oleifera seed is a legume and does not contain high content of carbohydrate as reported by Compaoré et al., (2011).

Conclusion

In conclusion, the results obtained in this study showed that high levels of MOM caused significant reduction in microbial load and increase of protein and moisture contents of smoked C. gareipinus. There was a general increase in microbial counts as storage progressed. The 4% MOM treated samples exhibited the highest antibacterial effect and was able to show the highest protein and moisture level. However, protein is the most important nutrient required from fish. The control samples had diverse microflora than other treatments. This study provides a possible utilization of M. oleifera seed in extending the shelf life of C. gariepinus.

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