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Storage and Microbial Evaluation of Black Pepper Pre-Treated Oven-Dried Moon Fish (Citharinus citharus Geoffery Saint-Hilaire 1809)

Agbabiaka LA[1]*, Kuforiji OA[2] and Ndumnigwe OE[3]

[1]Department of Fisheries Technology, Federal Polytechnic Nekede Owerri, Imo State, Nigeria
[2]Department of Agricultural Technology, Federal Polytechnic Ekowe, Bayelsa State, Nigeria
[3]Department of Fisheries Technology, Imo State Polytechnic, Umuagwo-Ohaji, Imo State, Nigeria

Abstract

The storage and microbial evaluation of black pepper (*Piper guineese*) pre-treated oven-dried moon fish (*Citharinus citharus*) stored at an ambient temperature were studied. Thirty six (36) freshly caught moon fish weighing between 850-900 g were purchased, killed, eviscerated and rinsed thoroughly under tap water and were divided into 3 treatments of 12 fish in each. The first treatment was immersed in 3% brine without black pepper extract served as control, the second treatment was soaked in mixture of 3% brine and 1.5% black pepper extract while the third sample was immersed in 3% brine and 3% black pepper extract tagged MFS_A, MFS_B and MFS_C respectively. Each treatment fish were soaked in the respective solutions for 30minutes prior to been oven- dried using gas as energy for 5hours at the temperature range of 80°C-90°C. After drying, samples were allowed to cool at room temperature in separately labelled clean trays and subsequently stored in the quality control room for 7 days to determine the storage and microbial characteristics. Processed fish samples were subjected to microbial analysis of which results revealed that the (control) MFS_A had the highest microbial count of 17.2 x 10^5 followed by 10.8 x 10^5 and 9.6 x 10^5 for MFS_B and MFS_C respectively. Also, the microbial analysis showed that MFS_A favour the growth of *Staphylococcus aureus*, while *Klebsiella* spp. and *Bacillus* spp. were identified for MFS_B and MFS_C respectively. These results therefore, indicated that the use of 3% brine with black pepper at 1.5% and 3% concentration could have caused reduction of microbial load of oven- dried fish and improve its shelf-life.

Keywords: Microbial; Moon-fish; Evaluation; Spices; Oven-dried; Shelf-life

Introduction

Fish is a major source of protein whose harvesting, handling, processing and distribution provide livelihood for millions of people as well as providing foreign exchange to many countries [1]. In Nigeria, fish is the preferred source of high quality animal protein compared to poultry, beef, mutton and pork. It is cheap and highly acceptable, with little or no religious bias, which gives it an advantage over pork or beef [2].

Inspite of the high demand for fish in Nigeria estimated at 2.66 metric tonnes annualy, only about 30% of needs are met through local production, the rest being imported [3,4]. Fish is highly perishable, being a high protein food with typically high levels of free amino-acids which microbes metabolize to produce ammonia, organic acids, ketoses and sulphur compounds [5]. Irreversible changes that occur as fish dies result in fish spoilage and eventually decomposition begins to occur [6]. About one- third of the world food production is lost annually as a result of microbial spoilage. In fact, microbial activity is responsible for spoilage of most fresh and of several lightly preserved seafoods [7]. Smoked fish and shellfish products can be a source of microbial hazards including *Listeria monocytogenes*, *Salmonella* spp., and *Clostridium botulinum* [8]. It has been reported that smoked fish samples from four local Markets in Kainji Lake area of Nigeria were dominated by gram- positive bacteria, potential pathogens, coagulase positive *Staphylococcus* and *Escherichia coli* [9].

Moon fish (*Citharinus* spp.) is a genus of lute fish from tropical species belongs to the family Citharinidae and are found in most habitats but they are particularly abundant in swamp, where they spawn during the flood season [10]. Their deep and flattened bodies earn them the popular name Moon fish. It is a highly nutritious fish when smoked, dried or cooked. To improve the consumer acceptability of fish and shelf life/storage quality of fish, there may be need to spice the fish [11]. A spice is a dried seed, fruit, root, bark or vegetable substance primarily used for flavouring, colouring or preserving food. Examples are ginger (*Zingibar officinale*), Black pepper *(Piper guineese*), cloves, turmeric, rosemary, thyme, basil, red peppers, and cinnamon among others.

Some researchers have evaluated the effects of spices on processed fish with high degree of success. They include Ethiopian/African pepper (*Xylopia ethiopicum)* (okada) and Calabash nutmeg (*Myrustica monodora*) [12], Onion [13], brine and ginger [11,14]. Black pepper is a flowering vine in the family *Piperaceae* cultivated for its fruits, which is usually dried and use as a spice and seasoning. It is popularly known as hot leave and it is widely consumed in some part of West Africa especially Nigeria and Ghana on account of its nutritional and medicinal properties [15]. Also the anti-parasitic, anti-microbial and anti-fungal activities of the leaf and seed of *Piper guineese* have also been reported [16,17]. This study therefore was aimed at determining efficacy of black pepper extract pre-treatment on microbial load and storage characteristics of processed Moonfish.

Materials and Method

Site of experiment

This experiment was carried out at the Department of Food Science

***Corresponding author:** Agbabiaka LA, Department of Fisheries Technology, Federal Polytechnic Nekede Owerri, Imo State, Nigeria, Tel: 083 431 501; E-mail: adegokson2@yahoo.com

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and Technology, Imo State Polytechnic Umuagwo-Ohaji Nigeria. Umuago-Ohaji lies between latitude 5° 17¹ and 5° 19¹ N, longitude 7° 54¹ and 6° 56¹ E. It is twenty six kilometres from the State capital (Owerri) on the Port Harcourt road.

Collection of samples

Thirty six (36) freshly caught Moonfish (*Citharinus citharus*) weighing between 850-900 g were purchased from Swale Market in Yenegoa, Bayelsa State, Nigeria. Some quantity of dried black pepper seeds were bought at Ekeonunwa Market in Owerri, Imo State.

Preparation of samples

Dried Black pepper seeds were ground into powder using kitchen grinding machine. The fish samples weighing between 850-900g were grouped into three treatments of 12 fish each. First batch was immersed in 3% brine without spice extract (control), second batch in solution of 3% brine and 1.5% black pepper extract, while the third batch was also immersed into the solution of 3% brine and 3% black pepper extract respectively for 30 minutes coded MFS_A, MFS_B and MFS_C. The spice extracts were obtained by soaking appropriate quantity of ground spice (black pepper) in water overnight and sieved accordingly. Thereafter, fish samples were removed and put into separate baskets and covered with muslin cloth to drain for 5 minutes. After this time, the fish samples were arranged into oven trays and allowed to dry at temperature of 80°C-90°C for 5 hours.

Processing techniques

Drying was conducted by using gas oven. The pre-treated fish samples were arranged on the oven trays and allowed to dry for 5 hours, during which turning over of the fish were done at interval to achieve uniformly dried product. Thereafter, the dried product were removed from the oven and arranged on trays and were allowed to cool at room temperature before weighing in order to determine the moisture loss. Samples were labelled accordingly and carefully stored for 7 days to check the effect of the spice on the shelf life and microbial load of the fish.

Microbiology

Media preparation

Nutrient Agar (NA), Eosin Methylene Blue (EMB) Agar and Potato Dextrose Agar (PDA) were used for the media preparation according to Cheesebrough (2000). 28 g of Nutrient Agar (NA), 31.2 g of Potato Dextrose Agar (PDA), and 17.28 g of Eosin Methylene Blue (EMB) Agar were measured out according to the manufacturer's direction. Thereafter, the three measured media were dispersed into 3 conical flasks and 1 litres of distilled water was added respectively and shook vigorously for proper mixing. The media were autoclaved for 15minutes at 120°C and cooled at room temperature respectively. After that, 20 ml of each of the media were poured into 12 plates, i.e., quadruplet per agar sample. Other instruments such as wire loop, petri dishes, pipette, and beakers were sterilized. In the preparation of Potato Dextrose Agar (PDA), broad spectrum antibiotic (Chloramphenicol) was added to prevent the growth of bacteria.

Serial dilution

Serial dilution was carried out according to Cheesbrough, (2000). Ten (10) fold serial dilution was made for each fish sample, 5 test-tubes were filed with 9ml of peptone water, 1g of the sample was dissolved and later transferred with syringe into assigned test tube (making it 10 ml) and thoroughly mixed; further sequential dilution were made by taking 1 ml from each of the 10 ml mixture into other test tubes respectively.

Culturing, incubating, colony count and identification

These methods were carried out according to Cheesbrough (2000). After the serial dilution, 1 millilitre of each sample taken from 2nd and 3rd (10^{-2} and 10^{-3}) test tubes were transferred to petri- dishes that have been appropriately labelled. The spread plate method was used for culture. 2-3 drops of the diluents sample were dropped in each of the media and a bent glass rod dipped into ethanol and sterilized in an open flame was used to distribute the dropped samples in the media evenly and was repeated for other samples. The plates for bacterial count were kept on laboratory bench and allowed for 24 hours, while that of fungi and coliform were kept for 48 hours at room temperature. Thereafter, the bacterial count was done and the colonies that appeared as clusters in each plate was counted and recorded. Similar counts were done on fungi and coliform. The numbers were counted and recorded; identification was carried out using standard product with biochemical tests such as Gram Staining Techniques, Catalase Test, Motility Test, Indole Test, Citrate Test, Methyl-Red and Vogues Proskaur according to Cheesebrough [18].

Microscopic examination of microbes

The traditional method in the microscopic examination of bacteria in the laboratory is the grams staining method. The description of the staining method was extracted from Cheesebrough [18], while the method of the microscopic study of fungi was conducted according to Harrigan and Mclance [19].

Procedure of the gram staining

The Gram stain is basically four step involving water rinses after each step. The smear was air dried and gently heat fixed.

Flood the slide with crystal violet (30 seconds) and wash with tap water.

Flood with Grams iodine (brown) for 30 seconds and wash with tap water.

Carefully decolorize with 70% ethanol for 10-15 seconds until the thinnest parts of the smear are colourless. Wash with tap water.

Flood with Safranin (red) for 30 seconds and wash with tap water. Thereafter, place it at the draining rack for drying before viewing under microscope.

Biological evaluation

i. Dressed Weight=Carcass Weight-Weight of Offal.

ii. Total Weight loss=live Weight/ Carcass Weight-Weight after smoking

$$\%\ weight\ loss = \frac{Total\ weight\ loss}{Live\ weight\ of\ fish} \times \frac{100}{1}$$

Statistical analysis

Statistical analysis was carried out using One-way Analysis of variance. The data obtained from proximate and sensory evaluation were subjected to Analysis of Variance and Mean Separation [20] using SPSS Window 17.0 Version of Inc., USA.

Results

Weight characteristics of spice pre- treated oven dried moon fish (*Citharinus citharus*)

Table 1 shows the weight characteristic of spice pre- treated oven-

dried moonfish (*Citharinus citharus*). The percentage weight losses of the black pepper pre-treated oven-dried moonfish were 65.11%, 69.15% and 67.53% for MFS_A, MFS_B and MFS_C respectively.

Total bacteria and heterotrophic fungi plate counts on black pepper pre-treated oven-dried moonfish

Table 2 shows the bacterial and heterotrophic fungi plate counts on black pepper pre-treated oven-dried moon fish. The Total Viable Count (TVC) are 17.2×10^5, 10.8×10^5 and 9.6×10^5 cfu/ml, the total coliform counts are 8.0×10^5, 7.4×10^5 and 3.2×10^4 cfu/ml for treatments A, B, and C respectively.

Cultural morphological features of bacterial isolate from spice pre-treated oven-dried moon fish

Table 3 shows the cultural morphological features of bacterial isolate for spice pre- treated oven-dried Moon fish.

Cell morphology and biochemical characteristics of bacteria isolated from spice pre-treated oven-dried *Citharinus citharus*

Table 4 shows the result of the cell morphology and biochemical characteristic of the bacteria isolate. The table also shows the organism that caused the spoilage on the spice pre- treated oven dried moon fish. The organisms include *Staphylococcus aureus*, *Klebsiella* spp. and *Bacillus* spp.

Morphological identification of the fungal isolates

Table 5 shows the result of the morphological identification of the fungal isolates. The identified fungal organism such as *Penicillum* spp., yeast and *Aspergillus* spp. are shown in the table below.

Samples	Live weight of fish (g)	Dressed Weight (g)	Weight after Total smoking (g)	Weight loss (g)	Weight loss (%)
$MFS_{(A)}$	845.66	716	295	550.66	65.11
$MFS_{(B)}$	860	725.67	265.3	594.7	69.15
$MFS_{(C)}$	815	691.67	264.6	550.4	67.5

Average weight loss (%) =67.26

Key

$MFS_{(A)}$ fish sample treated with 3% brine solution (Control)

$MFS_{(B)}$ Fish sample treated with mixture of 3% brine and 1.5% *Piper guineese*

$MFS_{(C)}$ Fish sample treated with mixture of 3% brine and 3% *Piper guineese*

Table 1: Weight characteristics of spice pre-treated oven-dried moon fish.

Sample	Dilution	TVC (cfu/ml) on NA	TCF (cfu/ml) on EMB	TFC (cfu/ml) on PDA
MFS_A	1×10^{-2}	TNTC	TNTC	*TNTC*
	1×10^{-3}	17.2×10^{-5}	8.0×10^5	4.8×10^4
MFS_B	1×10^{-2}	200	TNTC	TNTC
	1×10^{-3}	10.8×10^5	7.4×10^5	3.6×10^4
MFS_C	1×10^{-2}	TNTC	TNTC	TNTC
	1×10^{-3}	9.6×10^{-5}	4.2×10^5	3.2×10^4

KEY

$MFS_{(A)}$ Sample pre-treated with (3%) brine solution (control)

$MFS_{(B)}$ Sample pre- treated with mixture of (3%) brine and 1.5 (%) black pepper extract

$MFS_{(C)}$ Sample pre-treated with mixture of 3% brine and 3% black pepper extract

TNTC: Too numerous to count

NA: Nutrient Agar

EMB: Eosin Methylene Blue Agar

PDA: Potato Dextrose Agar

TCF: Total Coliform Count

TFC: Total Fungal Count

TVC: Total Viable Count

CFU/ml (Colony Forming Units Per Millilitre)

Table 2: Total microbial counts on spice pre-treated oven-dried moon fish.

Samples	Media	Elevation Size	Dilution Shape	Chromogenese Prob.Org
$MFS_{(A)}$	NA	10^{-2}	Circular	Flat PunctiformYellow
$MFS_{(A)}$	NA	10^{-3}	Circular	Flat Punctiform Green
$MFS_{(A)}$	EMB	10^{-2}	Irregular	Flat Punctiform Pink
$MFS_{(A)}$	EMB	10^{-3}	Irregular	Flat Punctiform Pink
$MFA_{(B)}$	NA	10^{-2}	Circular	Flat 3mm Yellow
$MFS_{(B)}$	NA	10^{-3}	Circular	Flat 3mm Yellow
$MFS_{(B)}$	EMB		Circular	Flat Punctiform Cream
$MFS_{(B)}$	EMB	10^{-2}	Circular	Convex Punctiform Cream
$MFS_{(B)}$	EMB	10^{-3}	Visid	Flat 3mm Cream
$MFS_{(C)}$	NA	10^{-2}	Circular	Flat Punctiform Cream
$MFS_{(C)}$	NA	10^{-3}	Circular	Flat Punctiform Cream
$MFS_{(C)}$	EMB	10^{-2}	Circular	Flat Punctiform Cream
$MFS_{(C)}$	EMB	10^{-3}	Circular	Flat Punctiform Cream

Key:

$MFS_{(A)}$ Moonfish Sample pre-treated with (3%) brine solution (control)

$MFS_{(B)}$ Moonfish Sample pre- treated with mixture of (3%) brine and 1.5 (%) black pepper extract

$MFS_{(C)}$ Moonfish Sample pre-treated with mixture of 3% brine and 3% black pepper extract

NA=Nutrient Agar

EMB=Eosin Methylene Blue Agar

Prob. Org.=Probable Organism

Table 3: Cultural morphological features of bacterial isolate.

Sample Cell	Identified Morphology	Gram Reaction	Catalase Test	Coagulate Test	Citrate Test	Motility Test	Indole Test	Test Organism
$MFS_{(A)}$	Rod	-	+	-	+	+	+	Klebsiella spp.
$MFS_{(B)}$	Cocci	+	+	+	+	-	-	Staphylococus aureus
$MFS_{(C)}$	Rod	+	-	-	+	+	+	Bacillus spp.

$MFS_{(A)}$ Moonfish Sample pre-treated with (3%) brine solution (control)

$MFS_{(B)}$ Moonfish Sample pre- treated with mixture of (3%) brine and 1.5 (%) black pepper extract

$MFS_{(C)}$ Moonfish Sample pre-treated with mixture of 3% brine and 3% black pepper extract

Table 4: Cell morphology and biochemical characteristic of bacteria isolate from spice pre-treated oven-dried *Citharinus citharus*.

Discussion

The result of the weight characteristics of black pepper pre-treated oven-dried moon fish (*Citharinus citharus*) is presented in Table 1. The highest percentage weight loss was 69.15%, followed by 67.5% and the lowest 65.11% were recorded for MFS_B, MFS_C and MFS_A respectively. The average moisture loss (67.2%) from the oven- dried moonfish (*Citharinus citharus)* is in range with the value of 65.0% recommended by Cardinal et al. [21].

Table 2 showed the data of the total bacterial and heterotrophic fungi plate counts. The highest microbial count was recorded in MFS_A (17.2×10^5cfu/ml) while the lower microbial count were recorded in MFS_B and MFS_C (10.8×10^5 cfu/ml and 9.6×10^5cfu/ml) respectively. However, the results are in accordance within the safety limit ($\leq 10^5$) for total bacterial count for microbiological food [22].

It is also generally accepted that fish with microbial load less than 10^6 cfu/g is likely to be at the stage of being unacceptable from the microbiological point of view and unfit for consumption. The increase in the microbial count recorded in MFS_A (control) may be due to unnoticed improper hygiene, handling, storage and processing procedure

Samples	Macro culture	Microscopy	Identified organism
MFS_A	Growth form: powdery or value surface. Color: cream. Periphery: with five extension	Hyphae-septate conidiophores; rise vertically from hyphae,conidia born on the conidiophores in multi- link chains like a paint brush	*Penicillium* spp.
MFS_B	Growth form: round smut surface. Color: cream. Periphery: entire and raised	Blasto spores, numerous chlamydospores. round oval budding cells in chains	(Yeast) *Saccharomyces cerevisiae*
MFS_C	Growth form: velvety to flatty surface due to marked sporulation. Color: yellow	Hyphae: septate conidiophores borne laterally on the hyphae, non-septate numerous serigriateproced from the apical club-shaped sweepings conidia borne in chains on the sterigriate	*Aspergillus* spp.

Table 5: Morphological identification of the fungal isolates.

adopted. This is in agreement with the findings of Abologba and Iyeru [23] who reported that lack of proper smoking and hygienic handling of smoked fish product would result in a very high microbial load. It could also be as result of high moisture content of the smoked- dried product, enhancing the proliferation of these micro- organisms and this is in agreement [2] that improperly oven- dried fish samples may have a relatively high water activity level which is a pre requisite for microbial growth.

Furthermore, the lower microbial counts observed in MFS_B and MFS_C suggest that intrinsic factors which include physical, chemical and structural properties of the fish such as water activity, pH, available nutrients and natural antimicrobial substance and extrinsic factors such as storage time, temperature, humidity and the composition of storage atmosphere may have played a role [24]. The cultural morphology features of bacterial isolate from black pepper pre-treated oven-dried moon fish is presented in Table 3. After the isolation of the micro- organisms, the following organisms were identified. They include *Staphylococcus aureus*, *Klebsiella* spp. and *Bacillus* spp. These organisms were present because they are salt tolerant. The pathogens isolated from these samples were similar to the microorganisms reported for Smoked Catfish (*Clarias* spp.) in Benin Metropolis, Edo State, Nigeria [25].

Nevertheless, the occurrence of *Bacillus* spp. could be as a result of the prevalence of their spores in the environment most especially in the soil and could survive high temperature of fish processing [26]. *Bacillus* spp. causes a toxin- medicated disease rather than infection such as diarrhoea and emetic illness characterized by nausea and vomiting [26]. *Staphylococcus aureus* have been found to be relatively resistance to drying which is a property that favours their transmission from one host to another [27,28]. They also stated that they are able to grow in concentrations of sodium chloride up to 15%. The presence of *Staphylococcus aureus* might have been through handling as it is a normal flora of the skin [29]. In addition, Klebsiella species are found everywhere in nature. They can be found in water, soil, plants, insect, animal and human [30]. Klebsiella occurrence in MFS_B could be as a result of its prevalence on the environment especially in water.

Table 4 showed the cell morphology and biochemical characterization of isolate bacteria with cell morphology gram staining and biochemical characteristic such as Catalase test, Coagulate, Citrate, Motility test, Indole test were recorded. The morphological identification of the fungal isolates from black pepper pre- treated oven-dried moonfish is presented in Table 5. The identified fungal organisms were *Penicillium* spp., *Saccharomyces cerevisiae* (Yeast) and Aspergillus species. *Penicillium* spp. is a genus of Ascomycetous which is of major importance in the natural environment as well as food and drug production.

Penicillium species are present in the air and dust of indoor environment such as homes and public buildings. Penicillium species occurrence in MFS_A could be as a result of them having the ability to survive on salted food products [31]. Aspergillus species are highly aerobic and are found in almost all oxygen- rich environments where they commonly grow as moulds on the surface of a substrate, as a result of the high oxygen tension [32]. It may also be attracted by salt (brine) which is hygroscopic to provide the needed aerobic environment. Though, the microbial loads from this study generally are within the safety limit ($\leq 10^5$) for total bacterial plate count for microbiological food [22]. It is also generally accepted that fish with microbial load less than 10^6 cfu/g is likely to be at the stage of being unacceptable from the microbiological point of view and unfit for consumption. However, the microbial counts in this study are lower than the values (3.98×10^7 cfu/g and 1.65×10^7 cfu/g) reported for smoked catfish without spices at New Benin and Yanga Markets in Edo State, Nigeria respectively [25]. The higher microbial counts on fish from some of the markets may be likely due to a lack of proper smoking on the side of the fish processors or/and improper hygienic handling procedures adopted by the smoked fish sellers. This is in agreement with the findings of Abolagba and Iyeru [23] who reported that lack of proper smoking and proper hygienic handling of smoked fish products would result in a very high microbial load. Vincent [33] similarly reported higher microbial load on *Capsicum annum* procured from New Benin market in contrast to lower numbers obtained from Oba market. These differentials were linked with the higher human traffic and poor environmental sanitation of the New Benin market.

It was evident from this experiment that the concentration of spice extract (Black pepper) inspite of good/ hygienic handling procedures adopted greatly reduced the microbial load linearly from 7.2×10^5 cfu/ml in MFS_A sample without spice extract (control) to 10.8×10^5 cfu/ml and 9.6×10^5 cfu/ml for spice pre-treated samples (MFS_B and MFS_C) respectively; hence, black pepper extract is recommended for pre-treating fish prior to processing especially when smoked and/or oven-dried product is desired to enhance longer shelf life, consumers' safety and acceptability.

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