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Bacterial and Fungal Quality of Oven-Dried African Catfish (*Clarias gariepinus*) Marinated with Extracts of Sweet Orange (*Citrus sinensis*)

Akinjogunla, Victoria Folakemi[1*], Adefiranye, Oyetayo Olaoluwa[2,3]

[1] *Department of Fisheries and Aquaculture, Bayero University Kano, Nigeria.*
[2] *Floret Center for Advanced Genomics and Bioinformatics Research, Lagos, Nigeria.*
[3] *Food Security and Safety, Mahikeng Campus, North-West University, South Africa.*

*Corresponding author: Victoria Folakemi Akinjogunla; E-mail: vfakinjogunla.faq@buk.edu.ng; Phone: +234 806 8036 484

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Citrus sinensis generates a lot of waste that needs to be put into beneficial use. The study was done to investigate the shelf life of smoked *Clarias gariepinus* marinated in extracts of *Citrus sinensis*. Samples were taken for microbial analysis after the 3rd, 5th, and 7th days and compared with the samples not marinated. Microbial isolates were identified by conventional methods. The mean Total Heterotrophic Bacterial Counts (mTHBC) and mean Total Fungal Counts (mTFC) for the marinated samples ranged from 1.30 ± 0.3 to 3.20 ± 0.3 (cfu/g) and 1.2 ± 0.3 to 2.83 ± 0.1 (cfu/g), respectively, while the unmarinated samples had mTHBC values from 5.0–5.4 cfu/g and mTFC values between 4.8–5.1 cfu/g. The microorganisms isolated include *Escherichia*, *Salmonella*, *Shigella*, *Staphylococcus*, and *Vibrio*. While *Vibrio* spp., *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi* were capable of proliferating in both samples at different stages, yeast and molds were the only isolated organisms in the unmarinated samples. This showed that marination of oven-dried *Clarias gariepinus* with the extract of *Citrus sinensis* has enabled it to possess less microbial load by eliminating most of the potential pathogens of significant public health concern as well as extending the shelf life.

Keywords: *Clarias gariepinus*, oven-dry, biochemical test, microbial, smoked.

Introduction

The importance of fish and its end-products in the world cannot be over-emphasized as they are an excellent source of food (high-quality protein, essential fatty acids, vitamins, and minerals that play a crucial role in human nutrition) with an average consumption level of 20 kg/capita and serve as food security and a source of income to many inhabitants in riverine communities (FAO, 2016; Akinjogunla et al., 2017; 2021). However, fish and fishery products are highly perishable due to enzymatic autolysis, oxidative rancidity, and microbial spoilage, leading to quality deterioration and reduced shelf-life (Akinjogunla et al., 2023). Smoking of seafoods as a form of preservation has been recorded as an ancient and conventional method since time immemorial in many developing and under-developed countries (Kumolu-Johnson et al., 2010). About 70–80% of the marine and freshwater catches harvested locally are consumed in smoked form (Adeyeye & Oyewole, 2016). The African catfish, *Clarias gariepinus*, is among the most commonly cultured (both inside and outside its range of tropical and subtropical environments) and smoked finfish in Nigeria (Adewolu et al., 2008; Akinola et al., 2006; Olorunfemi et al., 2022).

The use of natural antimicrobials and antioxidants derived from plant sources has emerged as a promising strategy to improve the preservation and shelf-life extension of smoked fish products (Shah et al., 2014). According to Oluborode et al. (2010), plant materials commonly used for preservation of edibles include, but are not limited to, *Allium sativum* Linn (garlic), *Citrus sinensis* (sweet orange rinds), *Syzgium aromaticum* (cloves), and their addition to these edibles has enhanced the qualities of fishes during storage (Ozyurt et al., 2012).

Sweet orange (*Citrus sinensis*) is one of the most popular fruits in Nigeria, but very few consume the peel, which is arguably the healthiest part of the fruit (Barreca et al., 2011). Orange peel contains flavonoids that exhibit antioxidant, antimicrobial, anti-carcinogenic, antiviral, anti-allergic, and anti-inflammatory activities, even in higher quantities than the seeds. Orange peel in various parts of the world, including Nigeria, is available, if not free, at low cost (Debbarma et al., 2013).

Characterizing the influence of this natural plant-based marinade could provide an effective and eco-friendly preservation strategy to extend the shelf-life while maintaining the nutritional

value of this economically and culturally important smoked fish product. Microbial spoilage by bacteria and fungi represents one of the most significant challenges in stored fish products, leading to health hazards, nutritional losses, and reduced shelf life (Okpala et al., 2021). Studies across Nigeria (Akinjogunla et al., 2011; Udochukwu et al., 2016; Ayeloja et al., 2018; Akinjogunla et al., 2023) have frequently isolated *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, other coliforms, and *Aspergillus* components from both fresh and traditionally smoked seafoods, with some reports suggesting antibiotic-resistant profiles.

The aim of this research was to assess the microbial profile of the economically and commercially important African catfish, *Clarias gariepinus* (Burchell, 1822), marinated with sweet orange, *Citrus sinensis* (L. Osbeck, 1757) peel extracts.

Materials and Methods

Preparation of Fish Samples and Plant Materials

A total of 15 samples of the fresh African catfish, *Clarias gariepinus* (C. gariepinus), were obtained from a private local fish farm in Kano State, having an average mean weight of 2.21 g and average mean length of 4.56 cm, while the orange peels were collected from sellers at the popular fruit market in Kano State, Nigeria. The fish were gutted and washed before placing them on wire mesh and allowed to drip naturally for 40 minutes. The orange peels were washed and shade-dried at room temperature. The dried peels were milled into powdered form. Three hundred grams (300 g) of the powdered plant (orange peel) was soaked in 150 ml of distilled water at room temperature for 24 hours and extracted according to the methods of Hegazy & Ibrahim (2012). The extract was filtered through a Whatman No. 2 filter paper to remove peel particles, and the washed catfish were marinated in the solution for 5 minutes (note that prolonged marination will give bitterness to the finished products). The marinated samples were then smoked using an oven-gas.

Laboratory Analysis

Bacterial and Fungal Analysis

Isolation of Microorganisms

Water (1 ml) and the flesh of *C. gariepinus* (1 g) sample were added into test tubes containing 10 ml of peptone water and shaken strongly to dislodge any attached bacteria or fungi. This mixture was serially diluted, after which 1 ml was transferred to plates containing agars. Nutrient agar, MacConkey agar, and Eosine Methylene Blue agar plates were inoculated and incubated for 24 hours at 37°C for total bacterial counts, while the inoculated Sabouraud Dextrose agar plates for total fungal counts (TFC) were incubated for 72 hours at approximately 28°C and kept at 4°C in the freezer for classification and characterization (Akinjogunla et al., 2023). The incubations were carried out in triplicate, and the results obtained were expressed as mean and standard deviation.

Identification and Characterization of Bacterial Isolates

The bacterial isolates were characterized and identified using colonial morphology, pigmentation, cell shape, Gram staining reactions, and conventional biochemical tests. The characteristics of the bacteria were evaluated using Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). Some of the bacterial isolates were further classified to species level using the Vitek 2 automated system (Biomeriux Inc., France).

Identification and Characterization of Fungal Isolates

Fungal isolates were characterized and identified based on their colonial growth patterns, morphological features (soma, nature of hyphae, pseudo-mycelium, and asexual reproductive structures), and microscopic and macroscopic examination of the growth on the SDA plates (Ijato et al., 2021).

Statistical Analysis

Statistical analysis was done with the aid of SPSS (Statistical Package for the Social Sciences) computer statistical software package (SPSS, 2019). The descriptive statistics such as mean and standard deviation were calculated.

Result

In this study, Figure 1 shows the results of total viable counts of both bacterial and fungal isolates of oven-dried smoked catfish marinated with *Citrus sinensis* extract and the unmarinated samples from day 3 to the end of the first week. The result of the mean total heterotrophic bacterial colony counts ranged between 1.30 and 3.2 ($\times 10^{-3}$ cfu/g), while the mean total fungal counts were from 1.2 to 2.83 ($\times 10^{-3}$ cfu/g). The highest values (4.8–5.1 $\times 10^{-3}$ cfu/g and 5.0–5.4 $\times 10^{-3}$ cfu/g) for mean total fungal count and mean total heterotrophic bacterial counts, respectively, were recorded for catfish samples not marinated.

Nutrient agar, MacConkey agar, and Eosine Methylene Blue agar were adopted in the isolation of bacteria from the oven-dried catfish (marinated and unmarinated) samples, after which biochemical characterization was carried out to help with the identification and characterization of *Vibrio* spp., *Staphylococcus aureus*, *Escherichia coli*, *Shigella* sp., and *Salmonella typhi* based on their metabolic properties as shown in Table 1 below.

Discussion

Seafoods (finfishes and shellfishes) are fragile fish which are predisposed to quick decomposition which is usually triggered or initiated following enzymatic reactions and bacterial breakdown of the fish tissues, especially in the tropical areas, characterized with high temperature (Nwachukwu & Madubuko, 2013). According to Anwar et al. (2008), Sweet orange (*Citrus sinensis*) is one of those fruits that are loaded with bioactive compounds (pectin, phenolic, alkaloids, tannins, and flavonoids), which have influential functional properties as growth promoters, immune stimulators, improve drying rate of fish and have antiviral and antibacterial effects against some foodborne microorganisms often associated with spoilage of foods and foodborne illness (Bukar et al., 2010; Debberma et al., 2013). Report by Abiodun-Solanke et al. (2018) showed that catfish treated with orange peels improved shelf-life of products by 120 days of storage while Acar et al. (2015) documented that oil and flavonoids obtained from orange rinds extractions boost *tilapia* species' resistance against microbes like *Streptococcus* spp.

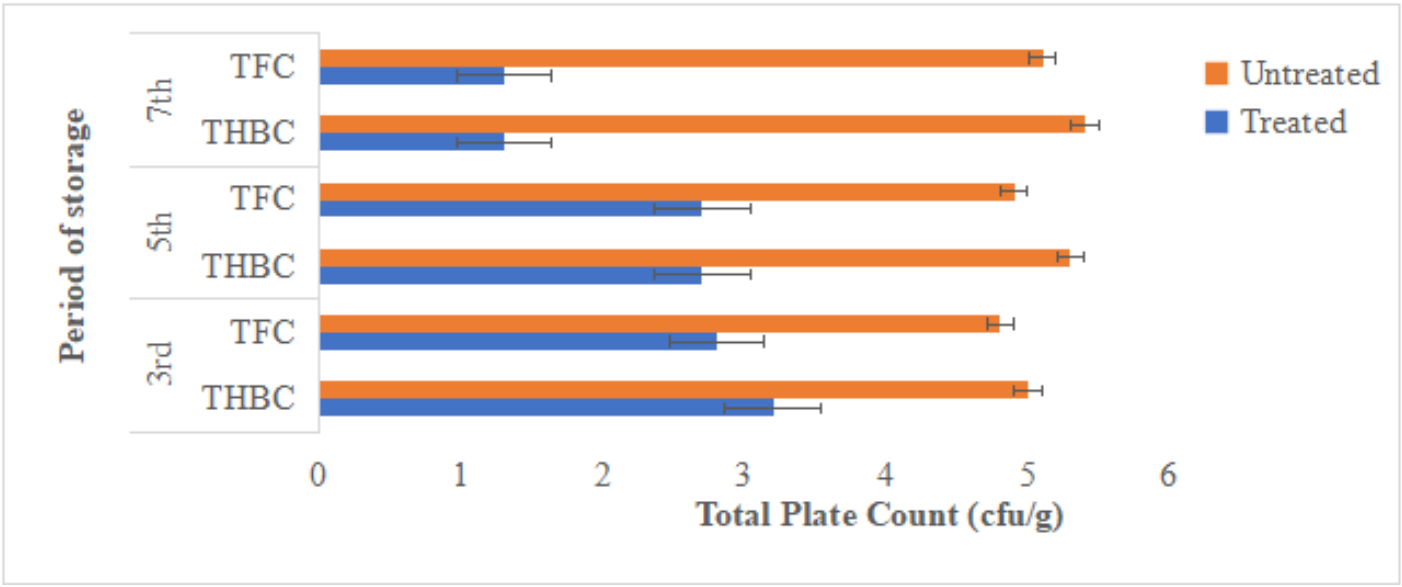

Figure 1 Bacterial and fungal counts of marinated oven-smoked catfish samples with *Citrus sinensis* extract.

Key: TFC – Total Fungal Count; THBC – Total Heterotrophic Bacterial Count; cfu/g – colony forming unit per gram of the fish sample.

Table 1 Morphological, biochemical, and enzymatic characteristics of bacterial isolates

Gram Reaction	CAT	VP	MR	IND	MOT	CIT	H_2S	OXI	ARD	ODX	ONPG	HYA	RAF	MAN	MAL	GAL	LAC	GLU	SUC	Probable Bacteria
+ cocci	+	+	+	-	-	+	-	+	+	+	-	+	-	+	+	+	+	+	+	*Staphylococcus aureus*
- c-rod	+	+	-	+	+	+	-	+	-	+	+	+	-	+	+	+	-	+	+	*Vibrio spp.*
- rod	+	-	+	+	+	-	-	-	-	-	+	-	-	+	-	+	+	+	+	*Escherichia coli*
- rod	+	-	+	+	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	*Shigella spp.*
- rod	+	-	+	-	+	-	+	-	-	-	-	-	-	+	+	+	-	+	-	*Salmonella typhi*

C-rod: Curved Rod; CAT: Catalase; VP: Vogues Proskauer; MR: Methyl red; IND: Indole; MOT: Motility; CIT: Citrate; H_2S: Hydrogen sulphide; OXI: Oxidase; ARD: Arginine Dehydrolase; ODX: Ornithine Decarboxylase; ONPG: Beta-galactosidase; HYA: Hyaluronidase; RAF: Raffinose; MAN: Mannitol; MAL: Maltose; GAL: Galactose; LAC: Lactose; GLU: Glucose; SUC: Sucrose; +: Positive; -: Negative.

In this research, evaluation of bacterial and fungal qualities of oven-dried African catfish (*C. gariepinus*) for a period of 7 days was studied to assess the presence of foodborne pathogens and the level at which they exist at different storage days after treatment with extracts of *Citrus sinensis*. The total plate counts for both bacteria and fungi did not exceed the range of specified microbiological limits (5×10^5 cfu/g; < 7.0 Log_{10} CFU/g and $\leq 10^6$ cfu/g) recommended for fish and fishery products (ICMSF, 2018; Health Protection Agency, 2009; Anihouvi et al., 2019) respectively. As the days progress, appreciable decrease in the microbial loads (bacterial and fungal counts) was observed compared to the untreated samples. The highest bacterial and fungal loads of 5.4 and 5.1×10^{-3} cfu/g respectively were found in the control (untreated) samples during analysis.

The THBC values of 3.2 Log_{10} cfu/g and 1.3 Log_{10} cfu/g at the 3rd and 7th day post-processed could be attributed to contamination of the raw material, poor handling, and storage. This post-harvest infection of smoked fish is in line with Kpodékon et al. (2014) and Anihouvi et al. (2019) who all documented that the level of microorganisms in fish reduces with smoking but increases with storage period and probably also during transportation. The presence of these organisms confirms microbial contamination either from poor smoking of the fish, poor environmental conditions, or packaging and storage of the fish. Also, the isolation of *Staphylococcus* in the smoked catfish sample may be attributed to post-processing contamination while *Vibrio* sp. isolation may be as an effect of unhygienic routine exercises or contamination from the water that the fish had been collected.

The presence of *Vibrio* sp., *Staphylococcus aureus*, *Escherichia coli*, *Shigella* sp., and *Salmonella typhi* as confirmed by the biochemical analyses carried out on isolates from all samples of oven-smoked catfish is in correlation with reports published by Akinjogunla et al. (2011; 2023) that isolation of pathogenic and spoilage organisms such as *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Aspergillus flavus*, etc., raises public health concerns about safety in consuming smoked fish products and causes a high rate of spoilage leading to shorter shelf/storage life of the product. Meanwhile, organisms

that cause foodborne diseases have been reported to include *Escherichia coli*, *Bacillus* species, *Clostridium botulinum*, molds, fungi, and yeast (Osakue et al., 2016).

Conclusion

Public awareness should be created, especially in the Northern parts of Nigeria, on food safety and the post-processing handling of smoked catfish products, with emphasis on proper fish packaging in well-ventilated baskets and transportation in properly sanitized trucks. The inclusion of a plant preservative (*Citrus sinensis*) has proven instrumental in reducing the microbial load of pathogens that are of public health concern. Based on the results confirmed in this work, it is recommended that catfish handlers be advised to embrace the use of sweet orange extracts in the marination of smoked fish to greatly reduce the microbial load of pathogens for safe consumption. Additionally, they should be enforced to use regulated heat or temperature in the preparation of wholesome and edible smoked catfish.

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