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Comparison of Microbial Profile of Sun Dried Fermented Tiger Fish (Hydrocynus ssp.) and Mud Fish (*Clarias anguiliaris*) Locally Known as Abil Alier Sold in Local Markets in South Sudan

Amegovu KA[1*], Mawadri M[1], Mandah J[2] and Yiga P[3]

[1]Department of Food Technology, College of Applied and Industrial Sciences, University of Juba, 82, Juba, Republic of South Sudan

[2]Faculty of Public Health and Management, International Health Sciences University, 7782, Kampala, Uganda

[3]Faculty of Science, Department of Food Technology, Kyambogo University, P.O. Box 1, Kampala, Uganda

Research Article

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***For Correspondence**

Amegovu K Andrewa, Department of Food Technology, College of Applied and Industrial Sciences, University of Juba, 82, Juba, Republic of South Sudan, Tel: +256772438219.

E-mail: kiri_andrew@yahoo.com

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ABSTRACT

Majority of South Sudanese are habitual consumers of fish, particularly the species of Tiger and Mud. Mostly, they are consumed in a sun dried fermented form. However, these two applied processing methods are still traditional with little quality control; fermentation is spontaneous while in addition to frequently fluctuating temperatures, sun-drying is done on bare ground introducing a number of hazards. Accordingly, these products pose a threat to public health. Literature points at a possible differential microbial profile across fish species, even when exposed to similar processing conditions, thus a differential risk profile. Hence, the need to compare the microbial profile of the two commonly eaten fish species in South Sudan to evaluate which of the two, presents a higher risk.

Methods: Sun dried fermented fish samples of both species were randomly selected from Konyokonyo market in the city of Juba. Standardized procedures based on International Standards Organization were used for the enumeration of microbial profile.

Results: Both fish samples had higher total microbial counts. However, Tiger Fish had comparatively higher counts than Mud fish, 3.7×10^6 cfu/g to 1.0×10^5 cfu/g compared to 3.0×10^4 cfu/g to 1.6×10^4 cfu/g for Mud Fish. Beneficial LAB formed the highest proportion of counts, 3.5×10^5 cfu/g to 4.1×10^4 cfu/g in Tiger fish and 1.5×10^4 cfu/g to 4.0×10^3 cfu/g in Mud fish. Enterobacteriacea, coliforms and molds were all present in levels at which they pose a risk to public health in both species, though c ounts were higher in Tiger Fish. Enterobacteriacea counts ranged from 4.0×10^3 cfu/g to 2.0×10^3 cfu/g in Tiger Fish compared to 2×10^2 cfu/g to 9.0×10^3 cfu/g on Mud fish samples. Coliform counts varied from 2.5×10^3 to 1.0×10^3cfu/g in Tiger fish and 9.0×10^1 to 1.0×10^1 cfu/g on mud fish samples.

Conclusion: Traditionally sun-dried fermented Tiger and Mud fish are microbiologically unsafe and pose a food poisoning risk to the public; undesirable enterobacteriacea, coliforms and molds are within risky levels. However, Tiger fish presents a much higher risk owing to its higher counts compared to mud fish. Post-harvest handling practices along the chain; harvesting to consumer consumption need to be aligned to the current good manufacturing practices.

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INTRODUCTION

South Sudan has a widespread network of waterways chief among them being the Nile River, its tributaries and the Sudd swamps. Fishing is thus a major activity in South Sudan; an estimated quarter (17.3%) of the population in South Sudan depends on capture fisheries as the main livelihood [1] and fish is a major component of the diet of most South Sudanese. Despite fisheries forming a substantial livelihood activity, fish processing is still traditional, done at household level under poor sanitary conditions and marketed through informal routes. No modern industries or improvement of classical rationale concerning fish processing has been established in South Sudan [1]. Additionally, the regulatory institutions are still weak.

In South Sudan, Tiger and Mud fish are the most commonly consumed fish species and are normally consumed in sun dried fermented form. Thus, main processing techniques are spontaneous fermentation and sun drying. Scientifically, drying and fermentation reduce water activity and increase acid levels to inhibit the growth of microorganisms [2]. However, this effect depends on the natural indigenous microbiota of the raw fish and the sanitary conditions of the processing environment [3,4]. Improperly done sun drying and fermentation can give rise to products which are a breeding source for pathogenic microorganisms, posing a food poisoning risk to consumers [2,5]. Thus, traditionally fermented fish could be a hub of several species of bacteria and yeasts [6-12]. Therefore, consumption of these products could put public at food safety risk. However, no risk profiling has been done in context of South Sudan on the commonly eaten sun dried fermented species of Mudd and Tiger fish. Studies show that even when exposed to the same processing conditions, different species may have significantly varying microbial levels/populations owing to the genetics and several other factors [13]. For example the feeding mechanism/mode ; Li found comparatively higher microbial levels in the gut of filter feeding fish species compared to those with grazing feeding habits [13]. Thus microbial levels on sun-dried fermented fish may vary across species. Thus the need to compare the microbial profiles of the two mostly eaten fish species in South Sudan [14]. This information is useful for the policy makers and consumers to evaluate which of the two species presents a higher risk.

MATERIALS AND METHODS

Sun-Dried Fermented Samples

Six random samples of each of these species (Mud and Tiger fish) were collected from Konyokonyo market in the city of Juba, South Sudan in April 2016 **(Figure 1)**. Fish processors were residents of Toj, an area where the river bank stretches outward or seasonal outflow swampy locations. The samples were collected in clean, dry containers and packed in sterilized polyethylene bags before being transported by road to Chemipher (U) Ltd; an internationally accredited food laboratory in Uganda for microbial analysis. The samples were analyzed immediately upon receipt.

Figure 1. Sun-dried fermented mud fish (left) & tiger fish (right) samples were of consistent size grade.

Microbiological Testing

Standardized procedures based on International Organization for Standardization, (ISO) were used for the enumeration of microorganisms.

Preparation of dilutions

Using ISO, serial dilutions were prepared before subsequent culturing by dissolving 1 Ringers tablet in 500 ml of distilled water. The solution was then filled in a 225 ml bottle for the first dilution and 9 ml screw capped test tubes for the subsequent dilutions. Sterilization was carried out at 121°C for 15 minutes and dilutions were cooled before use. Three replicates of each fish sample were weighed to 25 g and placed in a sterile stomacher bag. 22 ml of the sterile diluents were then aseptically added and blended in a stomacher for 3 minutes at 300 rpm. 1 ml of homogenate was added to a tube containing 9 ml of sterile dilutes. The vortex mixer was thoroughly mixed, and this was repeated for the third, fourth or more tube until the desired dilution was achieved.

Enumeration of microorganisms - colony count

Media powder was measured and dissolved in distilled water, autoclaved at 121°C for 15 min and cooled to 45-47°C in a water bath. Colony counts were obtained according to the International Standard [15,16]. 1 mL of each inoculum were aseptically added to the center of 3 Petri dishes. 20 mL of the molten agar were added to each of the petri dish. The inoculum were mixed

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with the agar and allowed to solidify. The Petri dishes were inverted and incubated at 37 °C for 24 hours. A control plate with about 20 mL of the medium was also prepared.

Using a colony counter, plates with counts ranging from 30-300 were counted and the average multiplied by the relevant dilution factor. Results were expressed as colony forming units per gram (cfu/g).

Enumeration of total coliforms - colony count technique

The International Standard [17] was used to enumerate total coliforms. The required media powder was weighed, mixed thoroughly with distilled water and heated until boiling with occasional stirring. The media was allowed to boil for 2 minutes and immediately cooled in water bath at 45-47 °C.

Using a sterile pipette, 1 mL of inoculum was transferred to the center of each Petri dish. 10 mL of Violet Red Bile Lactose agar were added into each Petri dish, mixed with the inoculum with the agar and allowed to solidify. A control plate with about 20 mL of the agar was prepared. The dishes were inverted and incubated at 30 °C for 24 ± 2 hours. The purplish colonies were considered as typical colonies of coliforms and didn't require further confirmation. Using a colony counter, counts ranging from 30-300 were taken.

To confirm the coliforms, medium powder was dissolved in distilled water. 10 mL of the medium was then dispensed in test tubes containing Durham tubes. Sterilization at 121 °C for 15 min was then applied. The Durham tubes did not contain air bubbles after sterilization. 5 colonies of each atypical type were then inoculated, into tubes of the broth and incubated at 37 °C for 24 ± 2 hours. Coliforms colonies that show gas formation in the Durham tube were then considered.

Enumeration of enterobacteriacea

Violet red bile glucose agar was prepared by mixing thoroughly the weighed media powder in distilled water, heating until boiling with occasional stirring for 2 minutes. The media was immediately cooled in water bath at 45-47 °C. 10 ml of agar were then added to each Petri dish containing 1 mL of inoculum. The agar and inoculum were mixed to solidify. 5 mL of the agar were then added onto the surface of the set inoculated agar and allowed to solidify. A control plate with about 20 mL of the agar was also prepared. The dishes were inverted and incubated at 37 °C for 24 ± 2 hours. The purplish colonies formed were considered as typical colonies of enterobacteriacea and using a colony counter, counts ranging from 30-300 were taken.

Enumeration of lactic acid bacteria

MRS agar was prepared by measuring the required amount of the media powder. The powder was then dissolved in distilled water, autoclaved at 121 °C for 15 minutes and then cooled to 45-47 °C in a water bath. 1 ml of inoculum was aseptically transferred to the center of each Petri dish to which 20 mL of the molten agar were added. The inoculum and agar were mixed by rotating and then the mixture was left to solidify. The Petri dishes were inverted and incubated at 37 °C for 24 hours.

The white colonies were considered as typical colonies of lactic acid bacteria and counts per dilution between 30 and 300 were obtained using colony counter. The microbial count represented as colony forming units per gram (cfu/g) were calculated as according to the formula [18];

$$C = \frac{\Sigma X}{V\,[n_1 + n_2\,(0.1)]\,d}$$

Where;

C is the number of microbial units (cfu/g)

ΣX is the sum of all the counted colonies

n_1 is number of Petri dishes at which the first counting was done

n_2 is number of Petri dishes at which the second counting was done

d is the dilution factor at which the first counting was done

V is the volume of the sample inoculated on the Petri dish

Enumeration of yeasts and molds- surface spread technique

Yeasts and Molds were enumerated as prescribed by International Standard [19]. Potato dextrose Agar (PDA) was used. To prepare the agar, the required amount of media powder was weighed and mixed thoroughly with distilled water. The medium was autoclaved at 121 °C for 15 min and immediately cooled in water bath at 45-47 °C. 1 mL of sterile lactic acid was added to every 100 mL of the sterile molten PDA. 20 mL of molten agar was aseptically poured on Petri dishes and left to set at room temperature. The plates were inverted to avoid condensed water from dripping back onto the solidified agar.

0.1 ml of inoculum was added onto the center of solidified PDA. The inoculum was spread evenly on the surface of the

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solidified PDA using a sterile spreader. The Petri dishes were incubated at 30°C for 3 days. The plates were incubated upright. A control plate of 20 ml of the medium was prepared. Colony forming units (cfu) were counted using a colony counter and the results were presented as cfu/ml.

RESULTS

Total Microorganisms in Sun-Dried Fermented Tiger and Mud Fish

With reference to Mud fish, Tiger fish had comparatively higher levels of total microbial counts. Samples of Tiger fish had microbial counts in the range of 3.7 × 10^6 cfu/g to 1.0 × 10^5 cfu/g compared to mud fish which had levels in the range of 3.0 × 10^4 cfu/g to 1.6 × 10^4 cfu/g **(Figure 2)**.

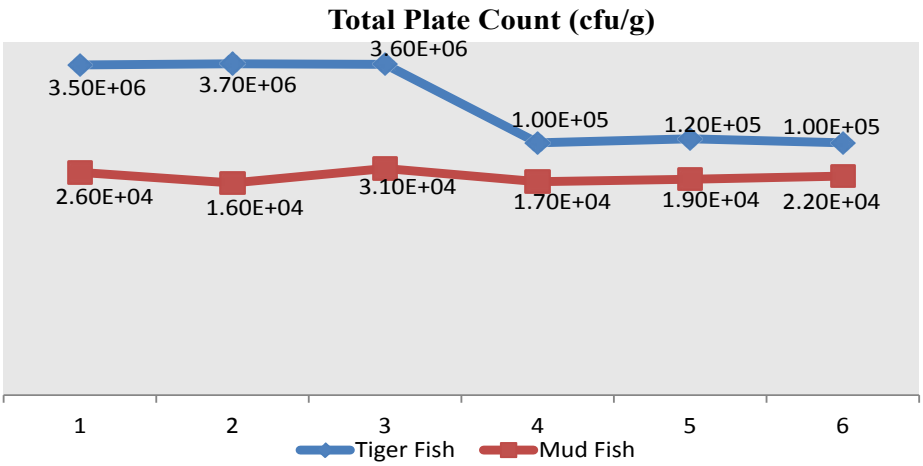

Figure 2. Total plate counts of microorganism of sun-dried fermented tiger and mud fish.

Lactic Acid Bacteria (Lab) in Sun-Dried Fermented Tiger and Mud Fish

Lower levels of LAB counts were found for sun dried fermented mud fish compared to sun dried fermented Tiger fish. Levels ranged from 1.5 × 10^4 cfu/g to 4.0 × 10^3 cfu/g across samples of Mud fish while for Tiger fish analyzed samples had LAB counts varying from 3.6 × 10^5 cfu/g to 2.10 × 10^4 cfu/g **(Figure 3)**.

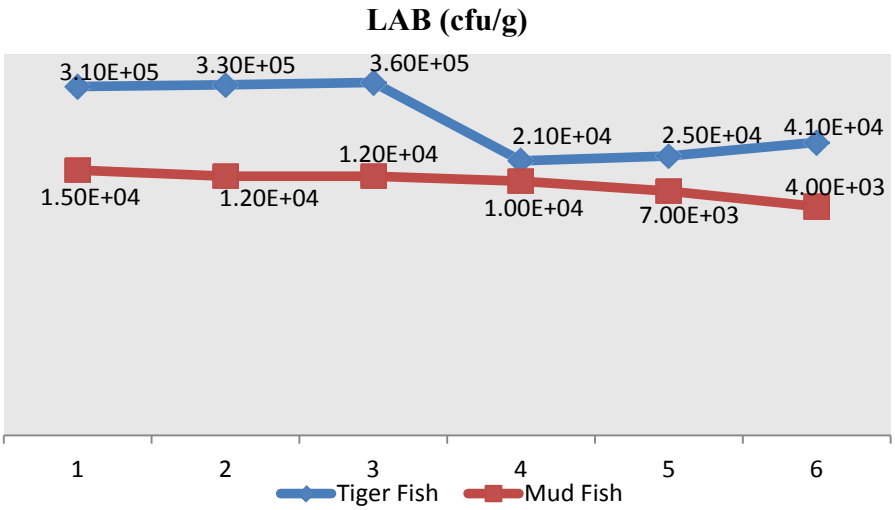

Figure 3. Levels of lactic acid bacteria in samples of sun-dried fermented tiger and mud fish.

Total enterobacteriacea in Sun-Dried Fermented Tiger and Mud Fish

Higher levels of total enterobacteriacea were found in samples of Tiger fish compared to Mud fish **(Figure 4).** Levels ranged from 4.0 × 10^3 cfu/g to 2.0 × 10^3 cfu/g across samples of Tiger fish compared to ranges of 2 × 10^2 cfu/g to 9.0 × 10^0 cfu/g on Mud fish samples.

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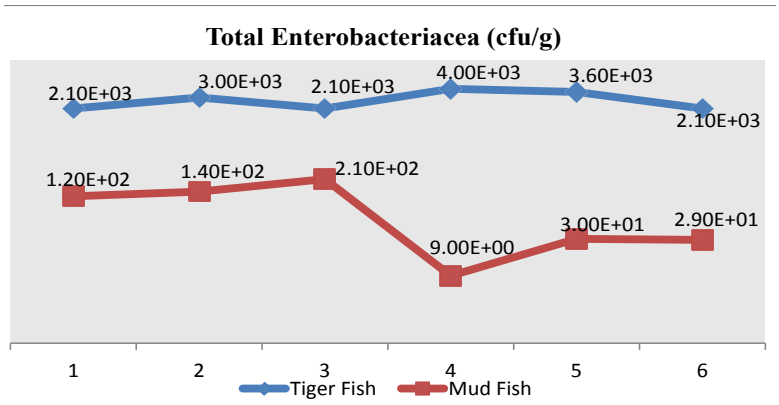

Figure 4. Levels of enterobacteriacea in samples of sun-dried fermented tiger and mud fish.

Total Coliforms in Sun-Dried Fermented Tiger and Mud Fish

Higher levels of total coliforms were found in samples of Tiger fish compared to Mud fish. Levels ranged from 2.5×10^3 to 1.0×10^3 cfu/g across samples of Tiger fish while counts ranged from 9.0×10^1 to 1.0×10^1 cfu/g on the six samples of sun dried fermented mud fish samples **(Figure 5)**.

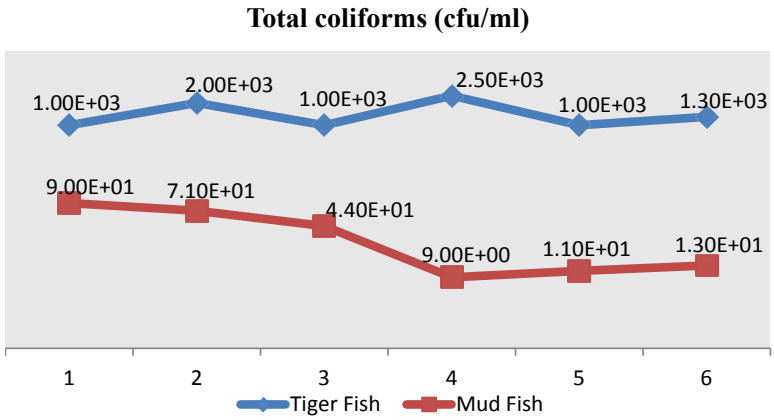

Figure 5. Total coliforms in samples of sun-dried fermented tiger and mud fish.

Yeasts and Molds in Sun-Dried Fermented Tiger and Mud Fish

Both fish species were contaminated with yeast and molds. However, Tiger fish had higher levels compared to Mud fish. Levels ranged from 2.0×10^2 cfu/ml to 2.5×10^2 cfu/ml across the six samples of Tiger fish compared to levels of 1.4×10^2 to 1.0×10^0 cfu/ml in the six samples of Mud fish **(Figure 6)**.

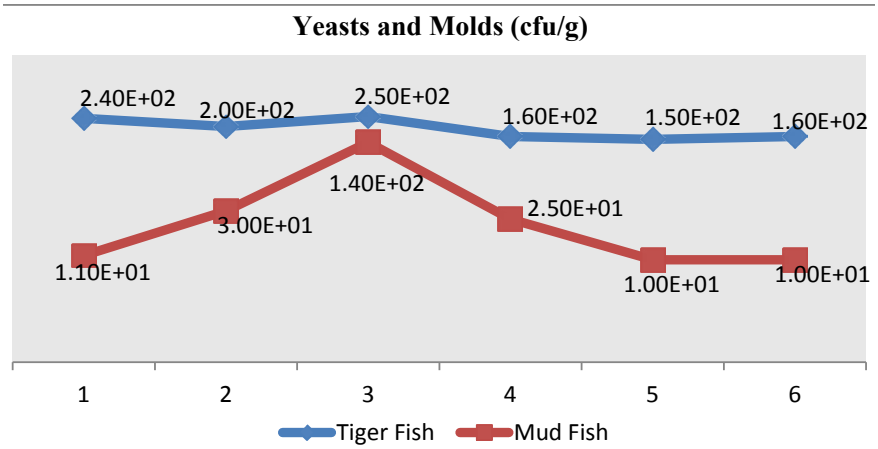

Figure 6. Total coliforms in samples of sun-dried fermented tiger and mud fish.

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DISCUSSION

Both fish species had a high total microbial count. However, Tiger fish had comparatively higher counts than Mud fish. Though, largest counts were of beneficial LAB, in both fish species harmful enterobacteriacea and coliforms were also present in levels at which they present a potential public health risk [20].

Like this study, a number of other studies [21,22] have reported fermented foods to be dominated by LAB species. Choun et al. [23] and Thapa et al. [10] have described fish fermentation to be mainly lactic acid based. Particularly, genera of *Alkalibacterium*, *Carnobacterium, Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus,* and *Weissella* have been reported [24-26]. LAB confers preservative as well as organoleptic desirable effects. Preservative effects include lowering the pH and producing a number of antimicrobial agents like bacteriocins and hydrogen peroxide. These preservative effects control growth of both spoilage and pathogenic microorganisms. Thus LAB species dominance roots from their creating undesirable proliferation conditions for other micro-organisms. Well as other microbes can't grow in such conditions, LAB species can proliferate well under them.

Though, Tiger fish had a relatively higher enterobacteriacea and coliforms levels, counts in both species were equivalent to those reported by Majumdar et al. [27] for shidal, a traditionally fermented fish in Northeast India. Through effecting decarboxylation reactions, using histidine decarboxylase, enterobacteriacea species are a major source of histamine in sundried fermented fish products [28-30]. Ingestion of 60 mg/100 g of histamine results in food poisoning. Fish is known to contain higher levels of free histidine in their muscle tissues, thus higher contamination with enterobacteriacea species avails histidine decarboxylase which releases histamine. Enterobactericea species not only present a histamine poisoning risk, but also species like *Salmonella*, *E. coli* O157 and *Shigella* have been reported across the globe, mainly in developed countries in serious incidences of food borne infections. In African countries, incidences maybe high, but usually go unnoticed because of gaps in data capturing. Found levels in both fish species were within the infective dose for most of these enterobacteriacea.

Generally, studies from other contexts, have reported *Staphylococcus aureus* and *Clostridium spp*. as the main coliform species on fermented fish [31,32]. In this study, no specie profiling was done but probably, the same species predominant. Different from other pathogenic bacteria, *Staphylococcus aureus* can grow at water activity levels as low as 0.87, thus improper drying introduces conducive conditions for its proliferation. Though the vegetative bacteria form is heat labile, usually denatured at cooking temperatures the sundried fermented fish are subjected to, the prior toxins they produce when they grow to sufficient numbers in a food are highly heat resistant. Therefore, food products contaminated with *Staphylococcus aureus* are a potent food intoxicant and a major public health risk.

Major source of enterobacteriacea and coliforms in traditionally sun dried fermented fish species could be the lack of control of processing conditions. First, fermentation is spontaneous and carried out in a hygienically inadequate environment which usually results in further contamination of the products instead. Secondly, sun drying does not yield an adequate water activity due to a number of factors, chief among them, being fluctuating temperatures. Sun drying on bare ground further makes the situation worse as soil is a known source of these undesirable enterobacteriacea species. At local markets, the storage facilities are poorly designed and are possible contamination route for these products.

Another important source of contamination is the aquatic environment where fish is harvested. Particularly, enterobacteriacea contamination in fresh fish has been associated with fecal pollution of water bodies [33,34]. The high initial contamination levels of freshly harvested fish is usually controlled by well-designed post-harvest handling practices following good manufacturing practices, however, in South Sudan, practices are inadequate to eliminate these contamination levels. Thus, as most fishing sources in South Sudan are highly contaminated, this may in one way also explain the high total microbial count found in this study.

Contrary to a study by Anihouvi, et al. [35] who did not find any yeast or mold on fermented cassava fish products, though in lower levels, in this study we found both fish species to be contaminated with yeasts and molds. Molds prevail at relatively lower water activity levels, thus considerable levels may be found in sun dried fermented fish products if good manufacturing practices are not followed. Mold spores are prevalent in air and soil, thus the unsanitary practice of sun drying fish on bare ground is a potential source of mold contamination as well. Additionally, storage practices in markets are inadequate, exposing the stored products to insects and mites, which are re-known sources of mold contamination through carrying spores on their bodies. These plus the temperature ranges of 25°C to 40°C of South Sudan which provide a conducive growth temperature may explain the mold levels found in this study. Previous studies in other settings, have reported commonly associated mold strains on sun dried fermented fish products to be *Aspergillus halophillus*; A. restrictus; *Wallemia sebi*; A. glaucus group; *A. candidus*; *A. ochraceus*; *A. flavus* and *Penicillum* spp. [36]. Though in this study, no profiling was done at strain level, there are higher chances that the same strains dominant as well on the studied fish species. Some of these strains produce mycotoxins which usually present both acute as well as chronic health effects depending on the concentration levels. Most problematic toxin is aflatoxin produced by *Aspergillus flavus* and *A. parasiticus*. Dangerous levels of aflatoxins have been reported in dried fish [37,38]. As mycotoxin levels particularly aflatoxin can be high in products even without any visible/organoleptic change, mold contamination poses a public health threat to fish consumers in South Sudan.

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CONFLICT OF INTEREST

The authors had no conflict of interest.

CONCLUSION

Both fish species were microbiologically unsafe; enterobacteriacea, coliforms and molds were present in levels at which they pose a food poisoning risk, particularly counts were higher in Tiger fish, showing that even when exposed to similar processing conditions, microbial profile varies across species. Unhygienic aquatic sources, unsanitary handling and processing procedures, mainly direct ground sun drying and storage at market places seem to be the source of contamination and need to be improved to reduce on the risk.

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