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Microbial profile of sun dried fermented mud fish (*Claria s. anguiliaris*) locally known as Abil Alier sold in local markets in South Sudan

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Abstract
Fish is a vital source of protein and PUFA's, widely consumed by the people of South Sudan. An estimated 143,000 tonnes of fish are harvested per year from the Sudd swamps and the Nile. However, fish processing is still traditional with no major improvements over the years. Traditional fermentation is the most widely used processing method. The process is often not well controlled, involves poor and unhygienic handling and storage practices, culminating in accumulation of microorganisms, jeopardizing consumer safety. Thus, this study characterized the microbial profile of sun-dried fermented mud fish widely consumed in South Sudan. Sun dried fermented mud fish samples were randomly selected from Konyokonyo market in the city of Juba, South Sudan. Standardized procedures based on International Organization for Standardization, (ISO) were used for the enumeration of microorganisms. The total microbial count of sun-dried fermented mud fish was high. Dominant microbial flora was lactic acid bacteria. Total coliforms, enterobacteriacea and yeasts and molds were present at potential food safety risk levels. Such results point at consumer safety issues, calling for improvements in the currently used classical processing and storage methods.

Keywords: fermentation, microbial profile, south Sudan, mud fish

1. Introduction
Fisheries contribute considerably towards food and nutritional security, particularly in the developing world. According to the Food and Agriculture Organization (FAO) (1997) [5], fish ranks high among the most important sources of quality protein, providing 16% of the animal protein consumed by the world's population. It is also an important source of essential fatty acids and micronutrients particularly calcium, iron, zinc and vitamin A (Kawarazuka and Béné, 2010) [12] that boast the immune function, infant growth and cognitive development (Metian, 2009) [15]. In South Sudan, fish is a vital source of food and income, with over 17.3% of the population deriving their livelihood from capture fishery alone (FAO, 2014) [6]. In 2012, 143,000 tons of fish valued at about USD 510 million were harvested, mainly from the Nile River, its tributaries and the Sudd swamps (Republic of South Sudan, 2013) [17]. Direct capture of fish increases household income and cash generated can also be re-invested into other activities (Kawarazuka and Béné, 2010) [12]. Hence, the fisheries sector is of socio-economic and cultural importance and could be the greatest contributor to food security, poverty eradication and improved nutrition in South Sudan.

However, neither modern industries nor improvement of classical rationale concerning fish processing have been established in South Sudan (FAO, 2014) [6]. Hence, traditional methods such as fermentation and sun-drying are widely used to process fish. These methods involve poor and unhygienic handling and storage practices. Very little control is exercised over the fermentation process leading to the growth of pathogenic organisms within a short period of time, especially considering that fish is a high risk food. Insufficient drying provides conducive breeding conditions (high water activity) for microorganisms (*Clostridium spp. Staphylococcus aureus*, and *Streptococcus spp.*) (Ahmed *et al.*, 2011). The fact that drying is carried out on bare ground, further exposes the products to microbial contamination as soil is a good source of a number of microorganisms (Ahmed *et al.*, 2011). Therefore, these processing methods to a certain extent present a public health food safety risk. However, in the context of South Sudan, there is little data, elaborating this risk, yet such information is of paramount importance to ensure consumer public health. Hence, this study assessed the microbial profile of sun-dried fermented mud fish widely consumed in south Sudan.

2. Materials and methods
2.1 Sun-dried fermented samples
Six random samples of sun-dried fermented mud fish were collected from Konyokonyo market in the city of Juba, South Sudan on 20th of April 2016 (Fig 1). Fish processors were residents of Toj. Toj is 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.

Fig 1: Sun-dried fermented mud fish Mud fish samples were of consistent size grade.

2.2 Microbiological testing

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

2.2.1 Preparation of dilutions

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

2.2.2 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 (International Organisation for Standardisation, 2003) [10]. 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 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).

2.2.3 Enumeration of total coliforms - colony count technique

The International Standard, (2006) 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. 10mL 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.

2.2.4 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. 10ml of agar were then added to each Petri dish containing 1mL of inoculum. The agar and inoculum were mixed to solidify. 5mL 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. 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.

2.2.5 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 (International Dairy Federation, 1991);

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

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

2.2.7 Enumeration of Yeasts and Molds- Surface spread technique

Yeasts and Molds were enumerated as prescribed by International Standard (International Organisation for Standardisation, 2009) [8]. 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 15min and immediately cooled in water bath at 45 - 47°C. 1mL of sterile lactic acid was added to every 100 mL of the sterile molten PDA. 20mL 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. 0.1mL of inoculum was added onto the center of solidified PDA. The inoculum was spread evenly on the surface of the solidified PDA using a sterile spreader. The Petri dishes were incubated at 30°C for 3days. The plates were incubated upright. A control plate of 20mL of the medium was prepared. Colony forming units (cfu) were counted using a colony counter and the results were presented as cfu/ml.

2.3 Statistical analysis
Data was analyzed using statistical computer software of SPSS version 11.5 (SPSS, Chicago, IL).

3. Results
3.1 Microorganisms - Colony count
The highest recorded microbial counts were $3.0x10^4$ cfu/g. Samples A, B, D, E, and F had considerably lower microbial loads compared with sample C (Fig. 2). B had the lowest microbial load, $1.6 x10^4$ cfu/g.

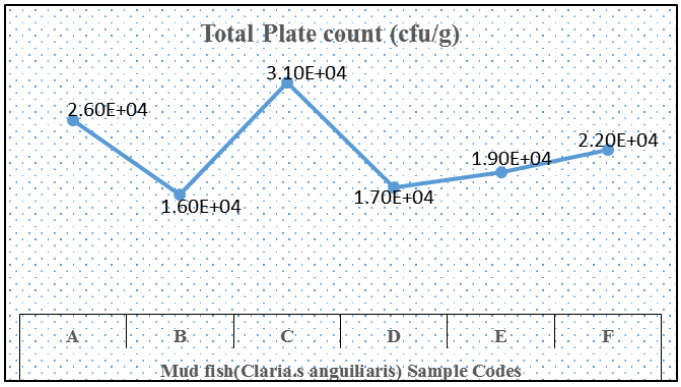

Fig 2: Total Plate Counts of microorganisms in samples of sun-dried fermented mud fish

3.3 Lactic acid bacteria (LAB)
LAB concentrations ranged from $1.5x10^4$ cfu/g in sample A to $4.0x10^3$cfu/g in sample F.

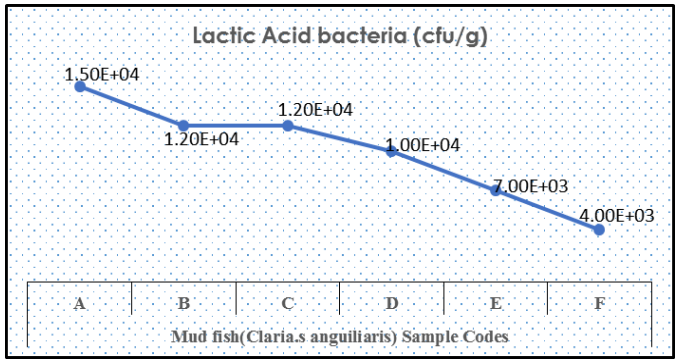

Fig 4: Levels of Lactic Acid bacteria in samples of sun-dried fermented Mud fish

3.2 Total coliforms
Total coliforms varied from $9.0x10^1$ to $1.0 x10^1$ cfu/g in the sun dried fermented mud fish samples (Fig. 3).

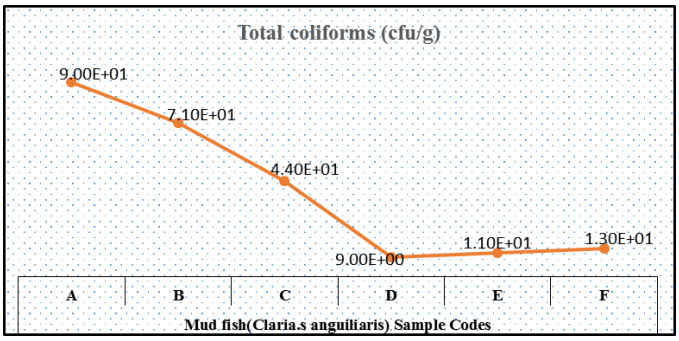

Fig 3: Total coliforms in sun-dried fermented Mud fish

3.4 Total Enterobacteriacea
Generally, the concentration of enterobacteriacea varied across samples. Levels ranged from as high as $2x10^2$cfu/g in sample C to as low as $9.0x10^o$cfu/g in sample D (Fig. 5).

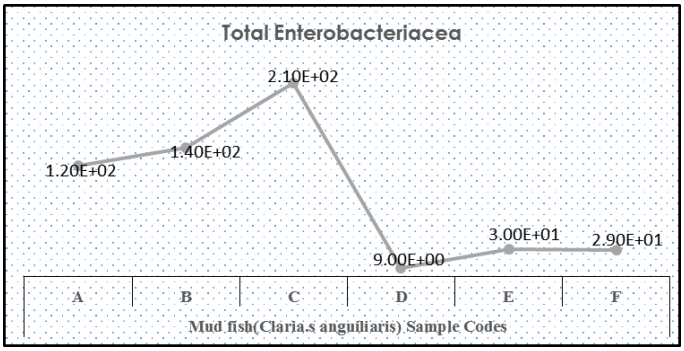

Fig 5: Levels of Enterobacteriacea in samples of sun-dried fermented Mud fish

3.5 Yeasts and Molds
Total yeasts and molds in samples of mud fish ranged from $1.4x 10^2$ in sample C to $1.0x10^0$ cfu/g in sample F (Fig. 6)

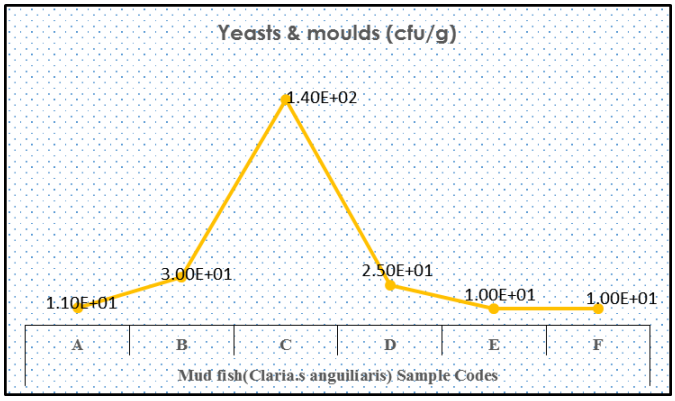

Fig 6: Levels of yeasts and molds in samples of sun-dried fermented Mud fish

4. Discussions
In this study, a high total microbial count was found. Generally, fermentation and sun drying lower water activity and pH inhibiting the growth of spoilage and pathogenic microorganisms. However, in line with this study, a number of studies across Africa have reported a higher microbial count in fermented sun-dried fish (Anihouvi *et al.*, 2007; Abd-Allah, S. M. S., 2011) [3, 1].
The high microbial count could be attributed to inadequate post-

harvest handling practices, from immediately after harvesting through processing to storage at the local markets. Very little control is exercised over the fermentation process, providing conducive conditions for growth of pathogenic micro-organisms. There are no institutional capacity/organization to perform quality control, processing and analysis on this processors (FAO, 2014) [6]. Higher contamination levels of the fishing water/environment usually translate in to a high microbial-flora on fresh fish (Kofi Manso, 1992) [14]. Since most fishing sources in South Sudan are highly contaminated, partly it may also contribute to the high total microbial count found in this study.

Despite the study finding a high total microbial count, total Coliforms, enterobacteriacea along with yeasts and molds were present in relatively lower levels. Enterobacteriacea concentration is highest in early stages of fermentation, after which it decreases probably due to decreasing pH (Zeng *et al.,* 2016) [19]. Therefore, it could be that most of the samples analyzed were in their concluding stages of fermentation, thus meaning lower levels of enterobacteriacea. Enterobacteriacea is a histamine forming bacteria, a component associated with food poisoning (causes allergenic like syndromes) especially when present in high levels (Sumitha *et al.,* 2014) [18]. Therefore, even when lower levels of enterobacteriacea were found, higher levels of histamine may already be present owing to production during earlier stages of fermentation when bacteria was still present in higher concentration, presenting a food safety complication to the consumers. Additionally, the found levels were within the infective dose for most bacterial microbes in the enterobacteriacea family like salmonella, E. coli O157 and shigella species.

Levels of LAB were particularly high. Such findings have been reported in fermented fish by Koffi-Nevry *et al.,* (2011) [13] in Côte d'Ivoire and Paludan-Müller *et al.,* (2002) [16]. The way fermentation process manifests makes LAB flora to be the dominant micro flora in fermented products. Through a sequentially dictated process, the LAB flora breaks down hexoses (carbohydrates) producing lactic acid which lowers the pH, further making conditions un-conducive for other micro-organisms, particularly bacterial populations *(*Paludan-Müller *et al.,* 2002) [16]. Hence, in any fermentation process, LAB is expected to be the dominant micro flora. In addition, to preservative effects, LAB confers detoxifying effects and improves nutritional value.

5. Conclusion

Total microbial count was high; however, the most dominant was LAB which has preservative effects. Never the less, although the enterobacteriacea, coliforms, yeast and mold levels found were low, their presence points at poor hygienic levels of these products, meaning food safety risk for consumers. Therefore, an improvement in the post-harvest handling practices including storage facilities at the local markets is needed to avert the risk posed by current practices.

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