International Journal of Food Science and Nutrition
ISSN: 2455-4898, Impact Factor: RJIF 5.14
Received: 07-10-2016; Accepted: 05-11-2016
www.foodsciencejournal.com
Volume 2; Issue 1; January 2017; Page No. 37-41

Microbial profile of sun dried fermented tiger fish (*Hydrocynus ssp)* locally known as Abil Alier sold in local markets in South Sudan

[1] Amegovu K Andrew, [2] Mawadri Michael, [3] Juliana Mandha, [4] Peter Yiga

[1, 2] Department of Food Technology, College of Applied and Industrial Sciences, University of Juba, Juba, Republic of South Sudan
[3] Faculty of Public Health and Management, International Health Sciences University, Kampala, Uganda
[4] Faculty of Science, Department of Food Technology, Kyambogo University, Kampala, Uganda

Abstract
Background: Traditionally, sundried fermented fish is a part of a habitual diet of South Sudanese. Sun-dried fermented tiger fish (*hydrocynus ssp*) is among the most consumed. Fermentation is usually spontaneous, largely uncontrolled and done under poor sanitary environments while sun drying is done on bare ground, possibly presenting microbial food safety risk. Despite this probable risk, no microbial profiling has been done on this commonly eaten sun dried fermented tiger fish. Hence, the need to characterize the microbial profile of sun-dried fermented tiger fish sold in local markets in Juba, south Sudan.
Methods: Sun dried fermented tiger fish samples 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 of samples.
Results: Analyzed samples had a high total microbial count, ranging from 3.7 x 10^6cfu/g to 1.0x10^5cfu/g. Beneficial LAB genera were the most dominant with counts in ranges of 3.5 x 10^5 cfu/g to 4.1 x 10^4cfu/g across samples. Though present in lower levels compared to LAB, total enterobacteriacea and coliforms were present in potential risk levels; 4.0x10^3 cfu/g to 2.0 x 10^3 cfu/g and 2.5x10^3 to 1.0x10^3cfu/g respectively. Yeasts and molds were also present, though in very low levels.
Conclusion: Sun-dried fermented tiger fish is microbiologically unsafe; though the beneficial LAB formed the highest microbial population, both enterobacteriacea and coliforms were within levels in which they pose a risk of food poisoning to the public. Efforts are thus needed to line the handling practices according to the good manufacturing practices so as to avert this risk.

Keywords: fermentation, sun drying, microbial profile, tiger fish

1. Introduction
Fish contributes to food security in many regions of the world, providing a valuable supplement for diversified and nutritious diets (Kawarazuka and Béné, 2010) [15]. It provides not only high-value protein, but also represents an important source of a wide range of micronutrients, minerals and essential fatty acids (Dincer *et al.,* 2010) [5]. With the abundant natural resources, such as rivers, swamps and wetlands in South Sudan, South Sudanese have become habitual consumers of fish. An estimated quarter (17.3%) of the population in South Sudan depends on capture fisheries as the main livelihood (FAO, 2014) [7]. The fisheries sector is thus of significant socio-economic potential. However, neither modern industries nor improvement of classical rationale concerning fish processing has been established in the country. Therefore, fish processing is mainly based on traditional methods of sun drying and fermentation.
Drying and fermentation reduce water activity and increase acid levels to inhibit the growth of microorganisms but this effect depends on the endogenous micro flora of the fresh fish and the sanitary conditions of the processing environment (El Sheikha *et al.,* 2014) [6]. With limited regulation and supervision, sun drying and fermentation can yield poor quality products that pose health risks to consumers (El Sheikha *et al.,* 2014) [6]. Products which are not insufficiently dried are a breeding source for undesirable microorganisms (Ahmed *et al.,* 2011). Particularly, these conditions are conducive for the growth of pathogens such as Staphylococcus aureus, fecal Streptococcus spp. and Clostridium spp. (Ali A, Ahmadou D, Mohamadou B, Saidou C, 2011) [1, 2]. Thus investigating and documenting microbiological profiles of locally processed fish and fish products is of paramount importance to avert food safety risks and ensure public health. To our knowledge, there is no study that has been carried out to document the microbiological profile of commonly eaten tiger fish in South Sudan. Hence, this study aimed at characterizing the microbial profile of sun-dried fermented tiger fish sold in local in markets in Juba, south Sudan.

2. Methods
2.1 Sun-dried fermented Tiger fish samples
Sun-dried fermented tiger fish locally known as samaga rubutu was obtained from Konyokonyo market in Juba City of South Sudan (Fig 1). Samples were randomly selected from the market on the 20th of April 2016. Samples were collected in clean, dry containers and packed in sterilized polyethylene bags. They were then transported to Chemiphar Limited; an internationally accredited food laboratory in Uganda for the microbial analysis. Analysis was done immediately upon receipt of samples.

Fig 1: Sun-dried fermented Tiger fish sold in local markets in Juba

2.2 Microbiological testing

The International Organisation for Standardization, (ISO) was used for the enumeration of microorganisms in the fish samples.

2.2.1 Preparation of dilutions

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 15min and cooled before use. 22ml of the sterile diluents were then aseptically added to 25g of each fish sample and blended in a stomacher for 3 minutes at 300rpm. 1ml of homogenate was added and thoroughly mixed. This was repeated for the third and fourth tube until the desired dilution was achieved.

2.2.2. Enumeration of microorganisms - Colony count

Colony counts were obtained according to International Standard methodology (International Organisation for Standardisation, 2003) [13]. Measured media powder was dissolved in distilled water, autoclaved at 121ºC for 15 minutes and cooled to 45 - 47°C. 1 mL of each inoculum was aseptically added to the center of 3 Petri dishes, thereafter, 20 mL of the molten agar were added to each Petri dish, mixed 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 methodology (2006) [12] was used to enumerate total coliforms. Measured media powder was 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 a water bath at 45-47 °C. 10 mL of Violet Red Bile Lactose agar were added into each Petri dish containing 1mL of inoculum. The inoculum was mixed with the agar and allowed to solidify. A control plate with about 20 mL of 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. 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 minutes 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 showed gas formation in the Durham tube were then considered.

2.2.4. Enumeration of Enterobacteriaceae

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 then immediately cooled in a water bath at 45-47 °C. 10mL of prepared agar were added to Petri dishes (3) containing 1mL of inoculum and 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 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 Enterobacteriaceae 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 and autoclaved at 121 C° for 15 minutes. The agar was then cooled to 45 - 47°C in a water bath. 1 mL of inoculum was aseptically transferred to the center of petri dishes (3) to which 20 mL of the molten agar were added. The inoculum and agar were mixed and left to solidify; thereafter 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 according to the formula below (International Dairy Federation, 1991) [10].

$$C = \frac{\Sigma X}{V\,[n1 + n2\,(0.1)]\,d}$$

Where;

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

ΣX is the sum of all the counted colonies

n1 is number of Petri dishes at which the first counting was done

n2 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

2.2.6 Enumeration of Yeasts and Molds- Surface spread technique

Yeasts and Molds were enumerated as prescribed by International Standard methodology (International Organisation for Standardisation, 2009) [11]. Potato Dextrose Agar (PDA) was used. To prepare PDA, the required amount of media powder was weighed and mixed thoroughly with distilled water. The medium was autoclaved at 121°C for 15minutes and immediately cooled in the 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 then 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 centre 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 20 mL of the medium was also prepared. Colony forming units (cfu) were counted using a colony counter and the results were presented as cfu/ml.

3. Results

3.1 Total microorganisms in sun-dried fermented Tiger fish (*hydrocynus sp)*

Total microbial count was high and varied considerably across the analyzed samples. Sample B had the highest levels, $3.7x10^6$ cfu/g while D and F had the lowest $1.0X10^5$ cfu/g (Figure 1).

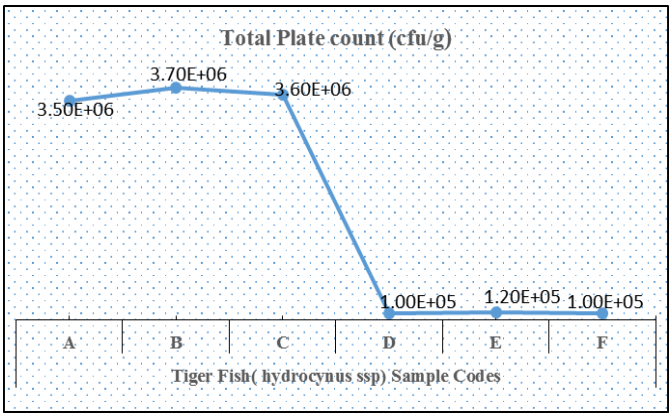

Fig 2: Total Plate Counts of microorganism of sun-dried fermented Tiger fish

3.2 Lactic acid bacteria (LAB) in sun-dried fermented Tiger fish (*hydrocynus sp)*

LAB content was high among all the selected samples of sundried fermented tiger fish. Counts ranged from $3.6x10^5$cfu/g to $2.1x10^4$ cfu/g.

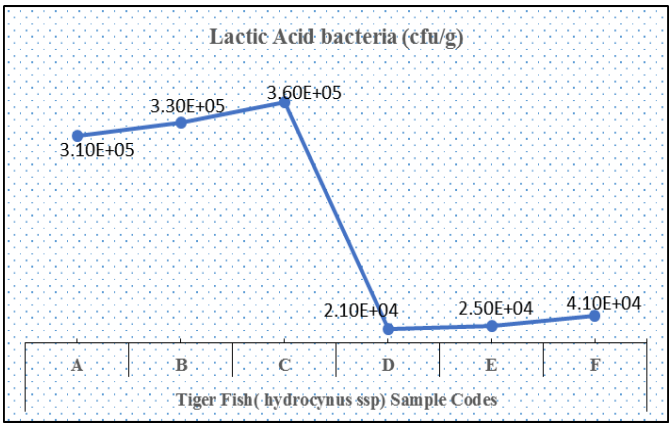

Fig 3: Levels of Lactic Acid bacteria in samples of sun-dried fermented Tiger fish

3.3 Total Enterobacteriaceae in sun-dried fermented Tiger fish (*hydrocynus sp)*

Total Enterobacteriaceae content was found to be high. Levels varied from $4.0x10^3$ cfu/g to $2.0X10^3$ cfu/g (Figure 4).

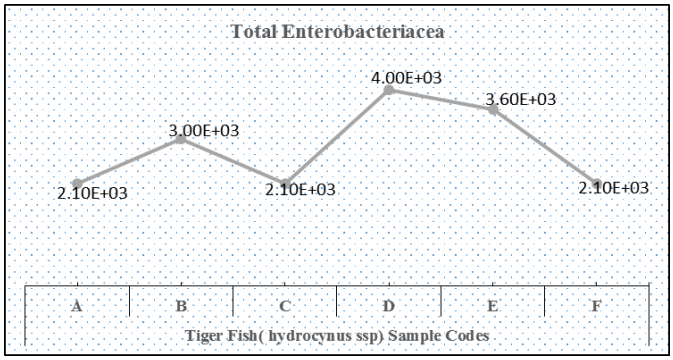

Fig 4: levels of Enterobacteriaceae in samples of sun-dried fermented Tiger fish

3.3 Total coliforms in sun-dried fermented tiger fish (*hydrocynus sp)*

Compared to enterobacteriaceae, total coliform levels were a little lower, ranged from $2.5 x10^3$ to $1.0 x10^3$ cfu/g, figure 5.

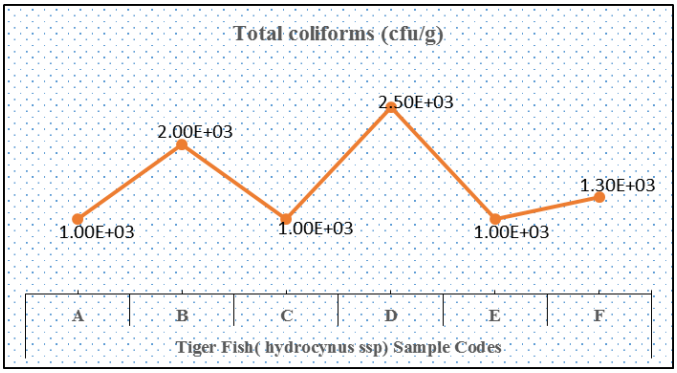

Fig 5: Total coliforms in samples of sun-dried fermented Tiger fish

3.5 Yeasts and Molds in sun-dried fermented Tiger fish (*hydrocynus sp)*

Fish samples were contaminated with yeast and molds. Levels ranged from $2.0x10^2$cfu/ml to $2.5 x10^2$cfu/ml, figure 7.

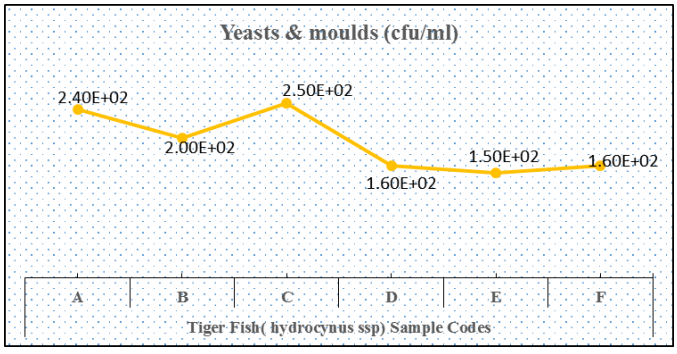

Fig 7: Levels of yeasts and molds in samples of sun-dried fermented tiger fish

4. Discussions

Total microbial counts were high. Predominant counts were the beneficial LAB. However, the undesirable enterobacteriaceae

and coliforms were also present in levels above those set in the legislation (ICMSF, 1986) [9]. Predominance of LAB in fermented fish products has been reported in a number of other studies (Chuon *et al.*, 2014; Thapa N, Pal J, 2004) [4, 20]. Fermentation of fish is highly lactic acid based. LAB genera of *lactobacillus, leuconstoc, streptococcus and pediococcus*, breakdown macro nutrients producing lactic acid and a number of desirable aromas and flavors (Salampessy, J., and Kailasapathy, K. 2010) [18]. In addition to lowering the pH, they also produce other antimicrobial agents like bacteriocins and hydrogen peroxide, limiting the growth of both the spoilage and pathogenic microorganisms. The preservative effects (low pH and antimicrobials) limit growth of other micro-organisms, but allow the thriving of LAB, explaining the high LAB count reported in this study.

Reported enterobacteriaceae and coliforms levels were comparable to those reported by Majumdar *et al.,* (2015) in his study of microbial profile of traditionally fermented fish called shidal in Northeast India. Enterobacteriaceae are capable of producing biogenic amines such as histamine (Lavizzari T *et al.*, 2010) [16] that are a risk factor of food poisoning. Additionally, bacterial microbes in the enterobacteriaceae family like salmonella, E. coli O157 and shigella species have been implicated in serious incidences of food borne infections. Reported levels were within the infective dose for most of these enterobacteriaceae. Also, coliform bacteria; staphylococcus aureus and clostridium spp. are reported dangerous food intoxicants in these levels.

Enterobacteriaceae and coliform profile of fish products usually results from two set of factors; contamination levels of the aquatic environment and the post-harvest handling practices including processing methods. In South Sudan, the fishing grounds are usually highly contaminated and this may explain the high microbial counts reported in this study. Particularly, enterobacteriaceae contamination in fish has been associated with fecal pollution of water bodies (Shabeeb *et al.*, 2016) [19]. 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, procedures are still so traditional with limited capacities to eliminate the contamination levels. The methods along with the processing environment are so inadequate in that they may be a source of additional contamination instead. For example fermentation is usually spontaneous with very little quality control measures exercised. Sun drying is associated with frequent temperature fluctuations, is carried out on bare ground, causing improper drying, providing a conducive water activity for growth and multiplication of microorganisms. Soil is a good source of both enterobacteriaceae and coliforms, hence, direct drying on soil introduces microbes on fish products. Storage conditions in local markets are also hygienically poor and usually provide conducive breeding conditions for micro-organisms. Therefore, these inadequate handling practices along the chain, especially during sun drying and storage (post fermentation), probably limit the preservative effects conferred by LAB, explaining the un usually high undesirable enterobacteriaceae and coliforms in these products. All these is escalated by the ineffective institutional abilities of mandated organizations to perform quality control, processing and analysis on these fish processors and marketers (FAO, 2014) [7].

Very low levels of yeasts and molds were found contrary to a study by Anihouvi *et al.* (2007) [3] who did not find any yeast and mold in fermented fish products. In addition to mycotoxin contamination, yeasts and molds cause various degrees of deterioration and decomposition of foods. They presence is an indication of unhygienic processing and storage conditions.

5. Conclusion

This study showed that sun-dried fermented tiger fish is microbiologically unsafe; though the beneficial LAB formed the highest microbial population, both enterobacteriaceae and coliforms were within levels in which they pose a risk of food poisoning. These could be a consequence of contaminated aquatic sources, unhygienic and uncontrolled post-harvest handling, principally post fermentation during sun-drying and marketing. Controlled post-harvest handling through applying good manufacturing practices during fermentation, sun drying (avoiding direct drying on soil) and storage improvements at local markets are needed to avert the risk posed to consumers of sun dried fermented tiger fish in the context of South Sudan.

6. References

1. Ali A, Ahmadou D, Mohamadou B, Saidou C, Tenin D. Influence of traditional drying and smoke-drying on the quality of three fish species (Tilapia nilotica, Silurus glanis and Arius parkii) from Lagdo Lake, Cameroon. Journal of animal and Veterinary Advances. 2011; 10(3):301-306.
2. Ali A, Ahmadou D, Mohamadou B, Saidou C, Tenin D. Influence of traditional drying and smoke-drying on the quality of three fish species (Tilapia nilotica, Silurus glanis and Arius parkii) from Lagdo Lake, Cameroon. Journal of animal and Veterinary Advances. 2011; 10(3):301-306.
3. Anihouvi VB, Sakyi-Dawson E, Ayernor GS, Hounhouigan JD. Microbiological changes in naturally fermented cassava fish (Pseudotolithus sp.) for lanhouin production. International journal of food microbiology. 2007; 116(2):287-291.
4. Chuon MR, Shiomoto M, Koyanagi T, Sasaki T, Michihata T, Chan S. *et al.* Microbial and chemical properties of Cambodian traditional fermented fish products. Journal of the Science of Food and Agriculture. 2014; 94(6):1124-1131.
5. Dincer T, Cakli S, Kilinc B, Tolasa S. Amino acids and fatty acid composition content of fish sauce. Journal of Animal and Veterinary Advances. 2010; 9(2):311-315.
6. El Sheikha AF, Ray R, Montet D, Panda S, Worawattanamateekul W. African fermented fish products in scope of risks. *I*nternational Food Research Journal. 2014, 21(2).
7. FAO (Food and Agriculture Organization). Fishery and Aquaculture Country Profiles. South Sudan. Country Profile Fact Sheets. In: FAO Fisheries and Aquaculture Department. Rome. 2014.
8. Frazier WC, westhoff DC. Microorganisms important in food microbiology, in: Food Microbiology. Mc Graw-Hill Book Company, New York. 1978, 539.
9. International Commission of Microbiological Specifications for Foods. ICMSF. Microorganisms in Foods 2- Sampling for Microbilogical analysis: Principles & Specific applications, 2nd ed. University of Toronto Press, Toronto, Ontario, Canada. 1986.

10. International Dairy Federation. International IDF Standard 100:B. Milk and milk products. Enumeration of microorganisms. Colony count technique at 30°C. 1991.
11. International Organisation for Standardisation. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 2: Colony count technique in products with water activity less than or equal to 0.95. Internationa Standard. 2009, ISO. 21527-2.
12. International Organisation for Standardisation. Microbiology of Food and Animal Feeding Stuffs: Horizontal Method for the Enumeration of Coliforms: Colony-Count Technique. International Standard. 2006, ISO. 4832.
13. International Organisation for Standardisation. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30C. Internationa Standard. 2003, ISO. 4833.
14. International Organisation for Standardisation. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection of Salmonella spp. International Standard. 2002. ISO. 6579.
15. Kawarazuka N, Béné C. Linking small-scale fisheries and aquaculture to household nutritional security: an overview. Food Security. 2010; *2*(4):343-357.
16. Lavizzari T, Breccia M, Bover-Cid S, Vidal-Carou MC, Veciana-Nogués MT. Histamine, cadaverine, and putrescine produced in vitro by enterobacteriaceae and pseudomonadaceae isolated from spinach. Journal of Food Protection®. 2010; 73(2):385-389.
17. Majumdar RK, Roy D, Bejjanki S, Bhaskar N. Chemical and microbial properties of shidal, a traditional fermented fish of Northeast India. Journal of food science and technology. 2016; 53(1):401-410.
18. Salampessy J, Kailasapathy KTN. Fermented fish products, in: Tamang JP, Kailasapathy K (eds) Fermented Foods and Beverages of the World. CRC Press, Taylor & Francis Group, New York. 2010, 289-307.
19. Al Shabeeb SS, Ibrahim MM, Al Ramadhan GH. A Comparative Microbial Quality Assessment among Fishes, Prawns and Cuttlefishes collected from Dammam Fish Market. Int. J. Curr. Microbiol. App. Sci. 2016; 5(9):405-418.
20. Thapa N, Pal J, Tamang JP. Microbial diversity in ngari, hentak and tungtap, fermented fish products of North-East India. World Journal of Microbiology and Biotechnology. 2004; 20(6):599-607.