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Microbial quality of Nile perch (*Lates niloticus*) and physico-chemical properties of salted sun-dried products sold at regional markets, Tanzania

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This study was conducted to investigate the microbial and physico-chemical qualities of salted sun-dried Nile perch (*Lates niloticus*) products and frozen fish from various storage facilities in Mwanza, Tanzania. The bacterial flora, moisture contents (MCs), and water activity (A_w) were investigated using standard methods. A total of 120 samples were collected for microbiological analysis with 90 of the samples additionally analysed for MC and A_w. Findings showed that the mean total viable counts (TVCs) in salted sun-dried products sampled during rainy season was 4.5 log cfu/g in fish heads with MCs of 38.0% and A_w of 0.682. This was significantly higher (P<0.05) than what was recorded during the dry season with mean TVCs of 3.0 log cfu/g at MCs of 24.6% and A_w of 0.625. Fish chests had TVCs of 3.3 log cfu/g and MCs of 27.6% and A_w of 0.659 in rainy season with no significant seasonal difference (P>0.05). Fish belly flaps had TVCs of 3.3 log cfu/g at 26.4% MCs and 0.669 A_w in rainy season which were comparable (P>0.05) to those dried in the dry season. The microbial species recovered were *Staphylococcus* spp., *Enterobacter* spp., *Psychrobacter* spp., and *Bacillus* spp. Neither *Escherichia coli* nor extended-spectrum beta-lactamase producing *Enterobacteriaceae* were detected. Frozen Nile perch had TVCs of 5.7 log cfu/g on skin, 5.4 log cfu/g in gills and 2.9 log cfu/g in flesh and were within acceptable limit set by Tanzanian standards. These results reveal that dried Nile perch products are generally safe for human consumption; however, the recovered bacteria indicate a need of implementing hygienic procedures during processing of products for improved quality and safety.

Key words: Salted sun-dried fish, microbial quality, food safety, physico-chemical parameters.

INTRODUCTION

Nile perch (*Lates niloticus*) from Lake Victoria is one of the most important fish species for fisher folks in Tanzania as well as for the nation due to its economic and nutritional health benefits (Kirema-Mukasa, 2012). Fish are an important source of animal protein and other essential elements to sustain human health (Ikwebe et al., 2017; Immaculate et al., 2013; Majumdar et al., 2017). Nile perch of good quality are processed as fillets

for export markets especially to the European countries and Asia while other fish parts are processed for domestic and regional African markets (Kabahenda and Hüsken, 2009; Kirema-Mukasa, 2012). Currently, the Nile perch market is growing due to product diversification including salted sun-dried bi-products such as heads, chests, belly-flaps and whole fish which are sold for human consumption. The salted sun-dried Nile perch products are mostly exported to countries such as the Democratic Republic of Congo, Rwanda and Burundi (Kirema-Mukasa, 2012).

Salting and sun-drying is an ancient preservation method which has been applied to different foods such as fish, meat, and vegetables (Immaculate et al., 2013; Nagwekar et al., 2017). Sun-drying of fish is simple, cheap, and affordable, but an adequate dried product requires enough sun (Ikwebe et al., 2017). The method can improve the shelf life of products if post-processing handling is properly done to avoid bacterial contamination (Nagwekar et al., 2017). Although the salted sun-dried Nile perch products have been widely marketed in East and Central African regional markets, limited information is available on their microbiological quality and safety aspects.

Moisture content (MC) and water activity (A_w) are important factors determining food quality, preservation and shelf life of food stuffs. Also, they are used to predict microbial growth and determine the microbiological stability of food products (Bevilacqua et al., 2017; Nielsen, 2010). Previous studies have described how the MCs and A_w can influence microbial growth on salted sun-dried fish and fish products (Nagwekar et al., 2017; Sampels, 2015). This includes the mechanism of products drying process which reduces the MC and Aw to minimise microbial proliferation in food. Although the preservation method is affordable, the drying condition, packaging, and storage may not be hygienically satisfactory to maintain the quality of the dried products.

Nile perch are caught in deep waters usually with low levels of microbial contamination (Immaculate et al., 2013; Koral et al., 2013). However, during subsequent handling along the value chain from capture to market, different bacteria of public health implications may come in contact with the fish, causing a decline in its safety (Immaculate et al., 2013). Therefore, the determination of microbiological quality of frozen Nile perch from cold storage facilities is very important as a strategy for safeguarding consumer's health.

The aim of this study was therefore to investigate the microbial quality and safety of frozen Nile perch and its bi-products in line with physico-chemical qualities of processed sun-dried products marketed in the Lake Victoria region.

MATERIALS AND METHODS

Sampling, laboratory sample preparation and analysis

A total of 120 samples were collected from March to July 2018. The samples included frozen Nile perch from cold storage facilities and salted sun-dried bi-product (heads, chests and belly flaps). Sampling locations were located in Ilemela and Nyamagana districts of Mwanza region, Tanzania. Microbiological and MC analysis were done at the National Fish Quality Control Laboratory (NFQCL), Mwanza and A_w analysis at the Department of Food Technology, Nutrition and Consumer Sciences laboratory, Sokoine University of Agriculture (SUA) in Morogoro. Identification of bacterial isolates was done at the Department of Veterinary and Animal Sciences, University of Copenhagen. Size of each sample was about 2 kg for frozen fish and 400 g of salted sun-dried products.

Ninety processed salted sun-dried Nile perch products were collected from different processors at the Kirumba Market in Mwanza. All samples were analysed for *Salmonella* spp., *Escherichia coli*, total coliform counts (TCCs), total viable counts (TVCs), and Extended Spectrum Beta-Lactamase (ESBL) producing *Enterobacteriaceae* using standard methods as described below. Samples were collected both in the rainy season (March-May) and dry season (June-July). Forty-five dried products (15 samples of each type) were collected during the rainy season and forty-five in the dry season (15 each sample type). Samples were collected using sterile rubber gloves, placed into sterile plastic zip-lock bags and transported to NFQCL for analysis. In the laboratory, each salted sun-dried sample was divided into three portions with the first portion used for microbiological analysis; the second portion was used for MCs analysis, and the third portion was packed and transported to SUA for the A_w analysis. For microbiological analysis, a 25 g sample was chopped and mixed into Buffered Peptone Water (BPW) (Oxoid Ltd, Hampshire, England) in sterile stomacher bags and homogenised in a stomacher (Seward 400, UK) before analysis.

A total of 30 frozen Nile perch were collected using sterile rubber gloves from storage facilities for microbiological analysis. Samples were placed in sterile plastic zip-lock bags, preserved in an insulated box containing cooling elements and transported to NFQCL for analysis. In the laboratory, frozen fish were thawed at room temperature for 2-3 h, and then by using sterile scissors and surgical blades; gills, skin and flesh were removed and chopped into a 25 g sample which was mixed with sterile 225 ml of BPW and analysed as per protocols stated in each method based on parameter analysed.

Detection of *Salmonella* species

Salmonella spp. was detected using the International Organisation Standard (ISO) method (ISO 6579:2002/Amd.1:2007). Briefly, pre-enrichment was done in BPW at 37°C for 24 h followed by enrichment in Rappaport Vassiliadis broth (Oxoid Ltd) at 42°C for 24 h and Mueller-Kauffman Tetrathionate-novobiocin broth (Oxoid

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Ltd) at 37°C for 24 h. Presumptive *Salmonella* colonies were biochemically confirmed on Triple Sugar Iron agar (Oxoid Ltd) and isolates were tested for agglutination with polyvalent *Salmonella* antisera (Rapid Lab Ltd, UK) with strain *S.* Typhimurium ATCC 13311 (Public Health, England) used as a positive control.

Enumeration of *Escherichia coli,* total coliforms (TCCs) and ESBL-producing *Enterobacteriaceae*

Enumeration of *E. coli* and TCCs were done on Brilliance *E. coli*/coliforms selective agar medium (BE/C) (Oxoid Ltd) by the spreading technique following the manufacturer's instructions. Serial dilutions were made according to ISO method (ISO 6887-1, 2017). From each dilution, 0.1 ml was drawn and inoculated onto prepared petri dishes containing sterile BE/C medium; the inoculum was spread, left to solidify, and then incubated at 37°C for 24 h. After incubation, bacteria were counted with the aid of a colony counter where colonies with purple colour were identified as *E. coli* while coliform bacteria were pink. *E. coli* ATCC 25922 was used as positive control. Enumeration of ESBL-producing *Enterobacteriaceae* was done on MacConkey agar (Oxoid Ltd), supplemented with 2 µg/ml of cefotaxime as described by Moremi et al. (2016).

Enumeration of total viable counts (TVCs)

TVCs were enumerated on plate counts agar (PCA) (Oxoid Ltd) at 30°C (ISO 4833-1, 2013). Serial dilutions were made as per ISO 6887-1 (2017), and from each dilution, 1 ml was drawn and inoculated into a sterile petri dish. The molten PCA was poured, mixed, left to solidify and the plate incubated at 30°C for 72 h. Colonies, that is, <300 colony forming unit (cfu) were counted with the aid of a colony counter. Colonies representing different morphological types were selected from the PCA plates and stored in 50% glycerol in liquid nitrogen for further analysis.

Moisture content analysis

The MC in salted sun-dried Nile perch products was determined according to the Association of Official Analytical Chemists Standard (AOAC) method number 950.46 (B) (AOAC, 2006). Briefly, 2 g of the sample (in duplicate) was weighed and evenly distributed into pre-heated petri dishes, then heated in an oven set at 102°C for 16 h parallel to an equal weight of pure pentahydrate copper sulphate ($CuSO_4.5H_2O$) as a control. Afterward, the sample was cooled in desiccator for 30 min and then reweighed. The average MCs (from the duplicates) were calculated and reported in percentage as per AOAC requirements.

Water activity analysis

The A_w was analysed according to the standard method (ISO 21807, 2004) using Novasina water activity meter (Pfaffikon, Switzerland). Briefly, a duplicate 2 g of grinded salted sun-dried sample was measured and placed into the water activity meter and left to stabilise for 20 to 30 min before the reading was recorded. The average of the duplicates was calculated and recorded as the final reading. Figures were reported with three decimals.

Microbial identification on salted sun-dried Nile perch products

The selected isolates from PCA were transported to Denmark for identification using matrix assisted laser desorption ionization-time-of-flight (MALDI-TOF) technology. The isolates were checked for purity following subculture onto blood agar and incubation at 37°C for 24 h, and then a single colony was selected and placed on glass slide for identification in Vitek MS MALDI-TOF Mass Spectrometer (bioMérieux, Inc., France). Identification of isolates was interpreted based on a comparison to SuperSpetra containing sets of genus, species and strains biomarkers characteristic for respective groups of microorganisms as stated in the instructions of the MALDI-TOF. Only isolates with an identification of 80% or more confidence was trusted.

Data analysis

Analysis of data was performed using Stata version 14 (StataCorp LP) descriptive statistics to obtain mean, standard deviation, and to show data variability in different parameters analysed. Also, the frequencies distributions of *Salmonella* spp. in different sample categories were determined. Seasonal variation in the different parameters analysed was analysed using single factor ANOVA. Results were presented in box-plots figures with the significance defined at $P<0.05$.

RESULTS AND DISCUSSION

Overall, high TVCs were reported in salted sun-dried Nile perch products collected during the rainy season with mean counts ranging from 3.3 to 4.5 log cfu/g while those collected during the dry season had lower mean counts ranging from 2.9 to 3.1 log cfu/g ($P<0.05$) (Figure 1a). However, these results are still within the acceptable limit set by the Tanzanian standard, that is, 1.0×10^6 cfu/g (TZS, 1988). The seasonal difference in TVCs was likely attributed to observed unhygienic products handling during, and after processing as well as drying conditions which attracted insects like flies on the dried products as also reported in previous studies (Immaculate et al., 2013; Nagwekar et al., 2017). The study shows the relationship between TVCs, MC, and A_w obtained in salted sun-dried Nile perch products collected in the rainy season and dry season, in that, the increase of MCs and A_w was proportional to the increase of TVCs in samples. The TVCs in Nile perch products were supported by the MC and A_w results obtained. High MC values ranging from 26.4 to 38.0% and A_w of 0.659 to 0.682 were recorded in products sampled during the rainy season when compared to products sampled during the dry season in which, MCs ranged from 18.3 to 24.6% and A_w 0.619 to 0.643, respectively (Figure 1b-1c). The average results of MCs and A_w were within the limits range required in salted dried fish and fish products (MCs 15-30%) and (A_w 0.60-0.75); but, were higher than the minimum limits for prevention of bacterial growth (MCs <15%; A_w <0.6) as specified in standards (IS, 2001; ISO, 1999). High TVCs, MCs and A_w values as seen in our products sampled during the rainy season are normally associated with high humidity, rainy weather conditions and low drying temperature (Logesh et al., 2012; Patterson and Ranjitha, 2009). Thus, the drying time

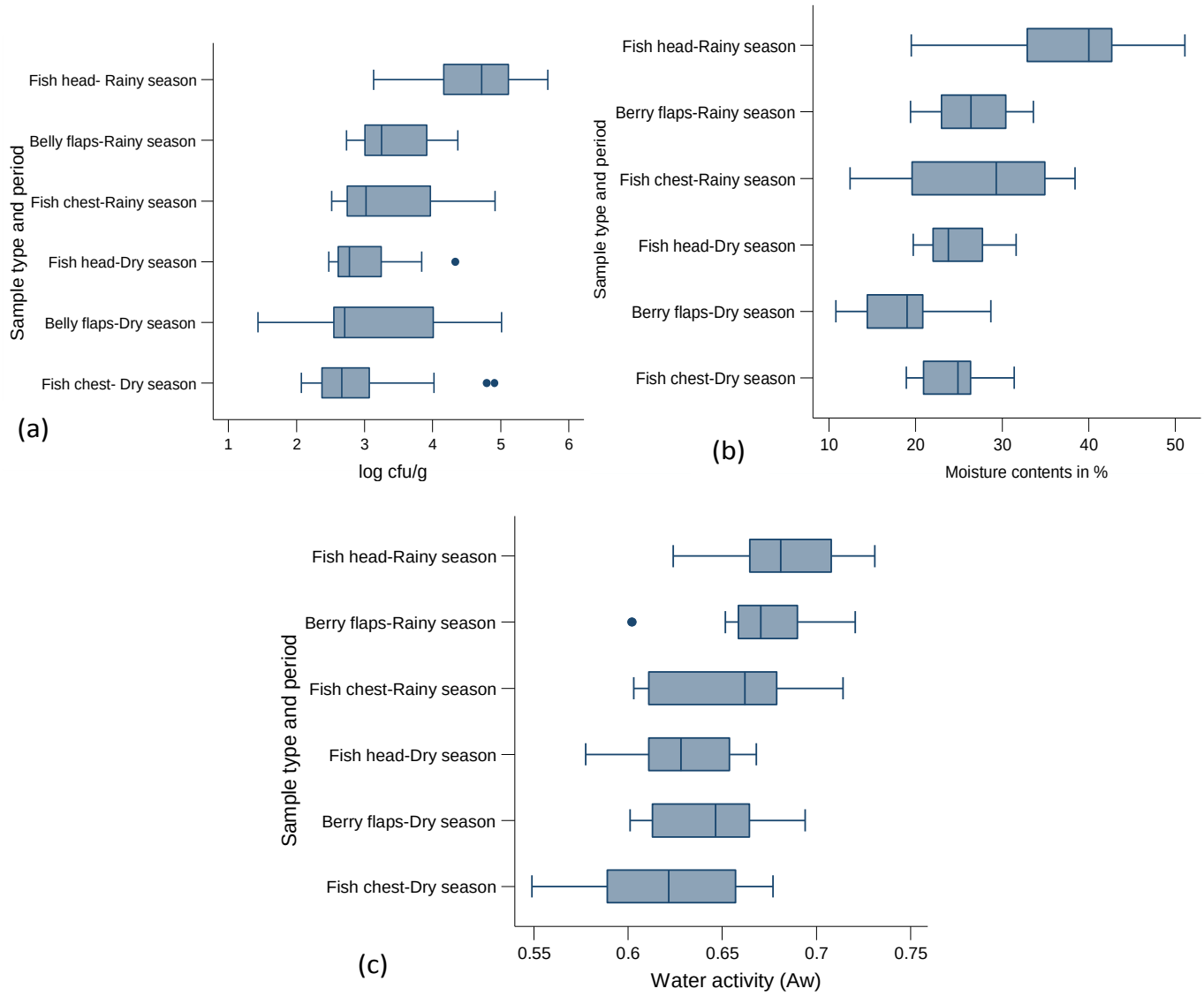

Figure 1. (a) Box plot of total viable bacterial counts in salted sun-dried Nile perch products, **(b)** Box plot of moisture contents (%) in salted sun-dried Nile perch products, (c) Box plot of water activity (A_w) in salted sun-dried Nile perch products.

during the rainy season is longer than in the dry season which together with poor hygienic conditions of processing premises might contribute to the products being more exposed to insect infestation and bacterial contamination (Ikwebe et al., 2017; Sivaraman and Siva, 2015). The study results are in agreement with other studies reporting high TVCs in salted sun-dried fish products (Nagwekar et al., 2017; Saritha et al., 2012; Sulieman and Mustafa, 2012). Also, MC values found in the current study were lower than the ones reported for different salted dried fish (Nuwanthi et al., 2016), but they were in agreement with the studies reported by Majumdar et al. (2017). The A_w values in this study were lower than 0.8 described by Koral et al. (2013), and 0.77 reported by Lin et al. (2012); irrespective of the season of sampling, however, they were higher than 0.5 reported by Geetha et al. (2014). Salt and drying processes are key factors contributing significantly to reduction of TVCs, MCs, and A_w in products (Ginigaddarage et al., 2018; Majumdar et al., 2017). The MCs and A_w values reported in the different salted sun-dried Nile perch products can be expected to support microbial growth during the rainy season so that products undergo microbial spoilage faster than those processed in the dry season.

Although the concentrations of bacteria suggest that the salted sun-dried Nile perch products are safe for human consumption, some of the identified bacteria especially *Staphylococcus xylosus, Bacillus megaterium*, *Klebsiella oxyota,* and *Enterobacter cloacae* might affect the products safety. These bacteria have been reported

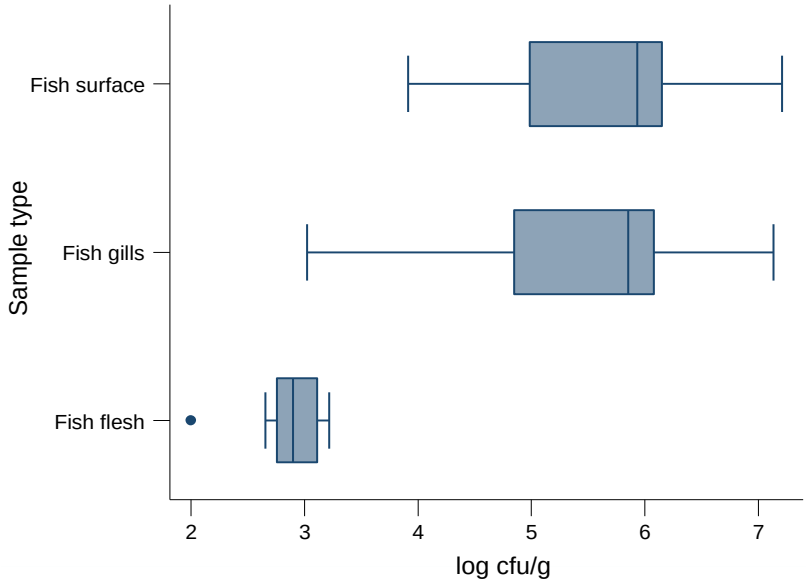

Figure 2. Box plot of total viable counts in frozen Nile perch samples.

in other studies to be responsible for histamine formation in salted fish and therefore pose a risk of histamine toxicity to humans consuming the products (Koral et al., 2013; Lin et al., 2012; Tsai et al., 2005). Histamine is a toxin formed by microbial decarboxylation of histidines as a result of time-temperature irregularity/abuse during storage of salted fish and/or fish products (Koral et al., 2013; Nagwekar et al., 2017; Tsai et al., 2005). The current study did not analyse histamine in salted sun-dried products; however, it is an area worthy of further studies in order to quantify the potential food safety risks to humans.

Our findings also shows that, only three out of 15 samples of belly flaps collected in rainy season had mean TCCs of 4.4 x 10^1 cfu/g and fish heads (1/15) had 1.4 x 10^1 cfu/g and *Salmonella* spp. was detected on the fish heads (6.7%, n=15) and, belly flaps (6.7%, n=15) samples collected during the rainy season whereas samples did not contain *Salmonella* spp. in the dry season. These findings are similar to those reported by Gabriel and Alano-Budiao (2015). Presence of TCCs and *Salmonella* spp. in dried products could be an indication of the poor products handling after processing leading to cross-contamination with bacteria from the environment. The absence of *E. coli* and ESBL-producing *Enterobacteriaceae* in the tested samples suggests that salted sun-dried Nile perch products were not contaminated with faecal bacteria.

Microbial load in fresh fish are an important determinant of the storage time of the products. The results of TVCs in frozen Nile perch showed high counts in skin and low in flesh (Figure 2). The high TVCs indicate that the fish can rapidly decompose and undergo spoilage when exposed to ambient temperature, as a result of metabolic activities. Moreover, *E. coli* concentrations were low where; 3/30 of fish gills had mean counts of 2.4 log cfu/g and skin (2/30) with counts of 2.1 log cfu/g while *E. coli* was not detected in flesh samples. The TCCs showed that fish gills (13/30) had mean counts of 2.8 log cfu/g whereas skin (7/30) had mean counts of 3.0 log cfu/g. The presence of *E. coli* and TCCs in samples albeit in low concentrations may imply poor fish handling as also described in other studies (Saritha et al., 2012; Sulieman and Mustafa, 2012) despite the fact that they were within acceptable limits as stated in Tanzania standard (TZS, 1988). The reported *Salmonella* spp. in fish gills (13.3%, n=30) and skin (6.7%, n=30) may suggest that the contamination may have occurred in the aquatic environment where fish were caught rather than from storage facilities.

Conclusions

The results of the present study provide important baseline information on the status of microbial quality in Nile perch products, which is essential for policy decisions geared towards safeguarding the quality and safety of these products to consumers. The different bacteria species recovered from salted sun-dried products provide an indication that there is a need for public authorities in the fisheries sector to recommend hygienic procedures to fit in salted sun-drying processing method.

Adoption of other drying methods that minimise contamination such as solar conduction dryers needs to be considered in order to preserve the quality and safety of Nile perch products in the study area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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