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Microbiological Quality of Traditionally Smoked Fish from Lake Victoria Crescent, Uganda

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Abstract

Microbiological quality of traditionally smoked fish was assessed to ascertain the effectiveness of traditional smoking process and handling conditions for smoked fish shelf-life and safety. Fish smoking is a popular preservation method in Uganda. Although fish smoking is a widely used method of preservation, the presence of spoilage and pathogenic bacteria in smoked fish and food borne illnesses remain a threat in developing countries. However, little is known about microbial quality of fish smoked from Uganda. In light of this, the status of spoilage, hygiene and pathogenic indicator organisms in fish (Nile perch) was investigated. A total of 75 randomly selected fresh, freshly smoked, and on shelf smoked fish from landing sites and markets respectively, were obtained for microbial and moisture content analysis. Descriptive statistics was used to present the data, while ANOVA and Tukey tests were used to analyze the data. The study revealed that, the smoking process significantly ($P < 0.05$) reduced microbial load in freshly fish to levels acceptable for consumption, despite the high levels of moisture content in the range of 59.2% to 66.5%. The mean microbial load (log of cfu/g) of indicator organisms for samples from different sites varied from; 9.97 to 10.18; 7.39 to 8.19 (aerobic mesophilic bacteria or TPC), 5.18 to 5.27; 0 to 4.28 (total coliforms), 2.73 to 5.55; 0 to 0.85 (*E. coli*), 4.97 to 7.32; 0 to 3.11 (*S. aureus*), and 2.21 to 4.56; 0 to 0.82 (Yeasts and molds), for fresh and smoked fish samples respectively. Additionally, mean microbial load for on shelf smoked fish from markets varied from; 8.2 to 10.57 (TPC), 2.8-6.63 (total coliforms), 0 to 6.11 (*E. coli*), 6.74 to 8.89 (*S. aureus*), and 0 to 5.51 (Yeasts and molds). Although findings showed that, traditional fish smoking was somewhat effective in reducing microbial load to acceptable levels, the microbial status of on shelf smoked fish indicated poor fish handling and storage. Therefore, proper fish handling during and post-smoking as well as cooking before consumption, are highly recommended for assured shelf-life and safety of smoked fish for consumption in Uganda.

Keywords: Microbiological, quality, smoked fish, Uganda, indicator organisms

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1. Introduction

Fish is a valuable resource because it is a source of cheap proteins, long chain n3 polyunsaturated fatty acids (LCn3PUFAs), and foreign exchange (Kleter, 2004; FAO/WHO, 2011) especially in developing countries (Egbal *et al.*, 2010). However, this food commodity is highly perishable, a factor that greatly affects its shelf-life and consumer safety. Fish perishability is attributed to its richness in essential nutrients that aid proliferation of microbes (Olaleye & Abegunde, 2015). Hence, fish would easily be rendered unfit for consumption within about a day after capture, unless its subjected to some form of preservation (Abolagba & Uwagbai, 2011). In light of this, various preservation methods such as smoking, sun drying, salting, freezing, and deep frying have been used to prolong the shelf-life of fish in many countries (Egbal *et al.*, 2010; Olaleye & Abegunde, 2015). Despite the existence of various preservations methods, most fish and other freshwater catch is consumed in smoked form. It is noteworthy that, practically all species of fish in most countries can be smoked (Adeyeye & Oyewole, 2016). Furthermore, fish smoking is the most preferred preservation method, because it imparts a desirable flavor and inhibits microbial growth, and thus prolongs shelf-life of smoked fish (Kumolu-Johnson *et al.*, 2009; Abolagba & Uwagbai, 2011). Coincidentally, this method is the most widely used for preserving fish in Uganda, with Nile perch (*Lates niloticus*) and Tilapia (*oreochromis niloticus*) fish species, being the frequently smoked by artisanal processors (Kabahenda *et al.*, 2009; Abigaba *et al.*, 2020).

Although fish smoking can destroy pathogens and spoilage organisms rendering smoked fish safe for consumption (Tzouros & Arvanitoyannis, 2000), some form of loss and spoilage can still occur especially with artisan processing or poor post-harvest technology (Kumolu-Johnson *et al.*, 2009; Abolagba & Uwagbai, 2011). Accordingly, the smoking method, the technique adopted and handling practices certainly influence both microbial safety and shelf-life of smoked fish (Salaudeen & Osibona, 2018; Tzouros & Arvanitoyannis, 2000). In view of this, high bacterial and mycofloral load in smoked fish beyond acceptable limits, have been reported in some countries. Their presence poses potential health hazards to smoked fish consumers, but also, compromises products' shelf-life (Adeyeye & Oyewole, 2016). Compromised shelf-life impacts on the availability of this cheap protein source for many consumers particularly the remote resource-poor population. Additionally, considering reports of

high spoilage and pathogenic microbial load in smoked fish from different countries, there was need to ascertain microbiological status of traditionally smoked fish from Uganda where information about the same, was largely scanty. Hence, this study was carried out in order to assess the microbial quality of fish smoked as a tool for surveying the effectiveness of traditional smoking process and the handling conditions, and measure of the shelf-life and safety of smoked fish consumed locally.

2. Materials and Methods

2.1 Study design and location

The study was carried out from Lake Victoria crescent of Uganda. Lake Victoria is the most important source of inland fishery production for human food and fishmeal industry. It is situated in East Africa, within 0°20'N to 3°00'S and 31°39'E to 34°53'E, at an altitude of 1134 m, and surface area of 68800 km² (Njiru *et al.*, 2008). Although Uganda's crescent of the Lake is approximately 43% (Njiru *et al.*, 2008), it contributes the largest proportion of the total national catch (UIA, 2009). Hence, the study area was considered based on; fish catch, popularity of the artisanal fish smoking technology among local fishing communities, market significance of smoked fish at the local and regional levels, and consumer base. For this study, a survey on quality of fish traditionally smoked from Lake Victoria, was carried out using fish samples obtained from; landing sites of Katosi, Kasenyi, and Ggaba (in Mukono, Wakiso, and Kampala districts respectively) and major markets of Bwaise, Nakulabye and Owino from Kampala district. Both processors of smoked fish and retailers were purposively selected based on their willingness to participate in the study and quantity of fish possessed.

2.2 Moisture content and fish smoking temperature determination

As part of evaluation for effectiveness of traditional fish smoking, moisture content of freshly smoked fish was measured to supplement the findings from the microbiological tests. Accordingly, moisture content for freshly smoked fish was obtained based on procedure by Swastawati, (2008). Briefly, approximately 5 g of fish sample was measured using a weighing balance (Sartorius AG, Gottingen/Germany) before heating and the weight was recorded as W₂ (wet weight). This was followed by heating in an air oven (WTB-Binder, Tuttlingen/Germany) at 105°C for 24 hours to obtain W₂ (dry weight), measured after cooling in a desiccator. Using the formula (1) below, moisture content for freshly smoked fish samples were calculated;

$$\% \text{ moisture content} = \left(\frac{W_2 - W_3}{W_2} \right) \times 100 \quad (1)$$

2.3 Microbiological analysis

2.3.1 Sampling and sample size

For microbiological analysis, five sample units (fish samples) were obtained from every processor according to established sampling plan (ICMSF, 1986). Accordingly, a total of 60 fresh and freshly smoked fish samples were obtained from processors. Additionally, a total of 15 smoked fish samples were randomly obtained from the markets for microbiological evaluation. The indicator organisms considered included; aerobic mesophilic bacteria, total coliforms, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), as well as yeasts and molds. All samples were aseptically and randomly selected from top, sides, middle and bottom of sampled lots, and immediately transported in sterile polythene bags on ice (0°C), to Makerere University laboratory for analysis.

2.3.2 Sample preparation and analysis

Sample preparation for microbiological analysis was done according to International Commission of Microbiological Specifications for Food (ICMSF, 1978). While ensuring asepsis, fish samples were removed from sterile bags and portions excised from different parts including the skin and muscle. Approximately 25g was added to 225 ml of 0.1% sterile peptone water (Laboratorios Conda, South Africa) in sterile stomacher bags for homogenization in a stomacher (Kalyx Biosciences Inc., Canada) at 12000 revolutions/minute for 1-2 minutes. A ten-fold serial dilutions of 1 ml of homogenate was also done for all samples up to 10⁻⁵. The aerobic mesophilic bacteria load (Total Plate Count) was obtained by inoculation of dilutions including homogenate (10⁰) on plate count agar (PCA, Himedia, India) using pour plate method (FAO, 1992). Inoculated plates were incubated at 37°C for 24 hours, followed by calculation of counts for a plate with a dilution between 30-300 colonies to obtain microbial numbers per gram of a sample. On the other hand, *S. aureus* enumeration was carried out using surface spread method, and its confirmation was done using coagulase test (FAO, 1992). Briefly, inoculation of homogenate (0.25ml) and dilutions up to 10⁻⁵ on Baird-Parker agar (Himedia, India) was done. Inoculated samples were incubated at 37°C for 24-48 hours, followed by selection of dilutions giving between 20-200 colonies for establishing the presumptive *S. aureus* load. For total coliforms and *E. coli* load, parallel tests were made using same prepared samples. Both homogenate and dilutions were inoculated on Violet Red Bile Agar (VRBA, Himedia, India) medium by pour plating and then incubated at 37°C for 24-48 hours. Pink colonies were counted on a dilution giving 30-300 colonies for calculation of total coliform load. The similar inoculated dilutions were instead incubated at 45°C for 24-48 hours on VRBA and pink colonies counted to estimate *E. coli* load (Harrigan, 1998).

Table 3: Mean microbial load of smoked fish from the markets

Microbial Parameter	Nakulabye (Log of cfu/g)	Bwaise (Log of cfu/g)	Owino (Log of cfu/g)	Recommended (Log of cfu/g)
TPC	8.20±1.02 ^{a*}	10.57±0.28 ^{b*}	8.40±1.56 ^{a*}	6-<7
TCC	2.80±0.59 ^a	6.63±1.11 ^{b*}	3.34±1.41 ^{a*}	2-<4
ECC	0 ^a	6.11±1.17 ^{b*}	1.59±0.56 ^{a*}	1.3-<2
STAPH	7.26±0.59 ^{a*}	8.89±0.27 ^{c*}	6.74±1.10 ^{a*}	1.3->2
Y&M	0 ^b	5.51±0.48 ^{a*}	2.84±0.66 ^a	<4

Key: Mean values with different superscripts (letters) in the same row, are significantly different, (P<0.05)
 Values with symbol * are above recommended limits
 TPC: Total plate count TCC: Total Coliforms Count ECC: *E. coli* count
 STAPH: *S. aureus* Y&M: Yeasts and molds

4. Discussion

Smoking involves heat application to remove water, and inhibit microbial and enzymatic actions on fish (Kumulu-Johnson *et al.*, 2010). According to Tzouros & Arvanitoyannis, (2000), the microbial stability in smoked fish depends on; salt level after brining, amount of heat applied (hot-smoking), the inhibitory actions of some smoke components, and the dehydrating effect of hot-smoking. From the study, moisture content somewhat reduced due to heating with the lowest value at 59.2%. However, even the lowest value achieved this was still higher than recommended 12% (Plahar *et al.*, 1999) for smoke-dried fish if a longer shelf-life is desired. Unsurprisingly, similar results were reported by Egbal *et al.*, (2010) in Sudan, because hot smoking technique used involves heating for a few hours which can hardly reduce the moisture content to as low as 12%. The high moisture content was presumed to favor microbial proliferation considering the water of activity effect on their growth. Accordingly, it was presumed that hot-smoked fish was still vulnerable to spoilage, and thus had a shorter shelf-life. It is noteworthy that, short shelf-life would affect smoked fish accessibility to the remote resource-poor population, who depend on this cheap product for essential nutrients (Adeyeye & Oyewole, 2016). Additionally, spoilage certainly affects socio-economic life of communities involved in artisan smoking sector, and the retailers involved smoked fish selling. In light of this, re-smoking to keep microbial numbers low, serves as a viable alternative for prolonging the shelf-life of hot-smoked fish “Muwotokwa” while on shelf.

From the study, both spoilage and pathogenic indicator organisms were isolated from fish samples and these included; *S. aureus*, Coliforms, *E. coli*, Fungi and generally mesophilic aerobic bacteria. Most of these are from soil, human, animals and untreated water (FAO, 1992; Ali *et al.*, 2011; Tegule, 2011). One of the important assays routinely used to predict the microbiological quality of foods is the enumeration of aerobic bacteria, and this is determined using aerobic plate count (APC) or total plate count (TPC) method (Diez-Gonzalez, 2014). Hence, the TPC was done to obtain a rough indication of microbiological quality of fish traditionally smoked from Uganda. Furthermore, the acceptable TPC load in fresh fish does not exceed 10⁷ cfu/g (ICMSF, 1986), but from this study, all fresh fish samples examined had aerobic mesophilic bacterial load above this value. This indicated poor microbiological quality of fresh fish from the processing sites. The high load was probably due to temperature abuse, poor personal hygiene, as well as water and equipment contamination. Although smoking process significantly reduced aerobic mesophilic bacteria, their numbers in freshly smoked fish (>7log cfu/g), was still higher than the recommended <4 log cfu/g (Gibert *et al.*, 2000). Our findings did not agree with the aerobic mesophilic bacteria load reported in Sudan (Egbal *et al.*, 2010), where zero counts for the same organisms was obtained. This was probably due to the differences in smoking technology and fish handling as well as initial microbial load in fresh samples before smoking. Similarly, our findings revealed slightly higher load than those reported in Ghana and Nigerian markets (Arthur & Osei-Somuah, 2004; Abolagba *et al.*, 2011). In addition, TPC for smoked fish obtained from markets was also more than the acceptable limits. The TPC status, indicated poor handling conditions, which required urgent attention from fish handlers and food quality agencies.

Furthermore, coliforms are among the indicator organisms whose presence especially in high numbers indicate a possibility of pathogenic organisms being present (FAO, 1992). Accordingly, coliforms were present in fresh fish samples from all landing sites with mean counts of over two fold above the acceptable limits (230 cfu/100g) by ICMSF, (1986). Essentially, the presence of high coliform load in fresh fish, is indicative of environmental contamination or pollution (ICMSF, 1986). Previously, a high load of coliforms (5 log of cfu/g) in water samples from Lake Victoria was reported, and this was attributed to industrial and domestic waste contamination (Tegule, 2011). In light of this, the differences in levels of total coliform load revealed by the study, could have been attributed to the varying levels of water contamination from different parts of the Lake and the general poor handling by fish handlers. Findings from a study conducted in Nigeria showed even higher coliforms count in fresh fish ranging from 7-8 log of cfu/g (Adebayo-Tayo *et al.*, 2012). Despite the high total coliform counts in fresh fish, traditional smoking process significantly (P<0.05) reduced coliforms to acceptable levels (Gilbert *et al.*, 2000), except for samples from Kasenyi landing site. The sensitivity of organisms to heating (Tzouros & Arvanitoyannis,

2000), could have been responsible for reduction of coliforms. Nevertheless, as indicators of hygiene and contamination (Gilbert *et al.*, 2000), high coliform load for Kasenyi samples indicated a likelihood of poor handling during smoking although the initial load in fresh samples could have also contributed. Furthermore, while the coliform load was within acceptable range for on shelf smoked fish, those from Bwaise market was not (Gilbert *et al.*, 2000). This pointed to re-contamination probably attributed to poor fish handling.

One of the most important members of coliforms is *E. coli*, and perhaps this is the most preferred indicator organism for contamination and mishandling of fish (FAO, 1992). In this study, presence of *E. coli* was investigated as measure for sanitary and health hazard monitoring. Accordingly, presence of higher *E. coli* levels about five fold more than acceptable 2 cfu/g (ICMSF, 1986), pointed to a possibility of fecal contamination from water and the fish handlers. This could have been fueled by increasing human settlement near the lake, lacking latrines but also being involved in activities generating industrial wastes that end up in the lake. Contamination of lake water with *E. coli* was reported in a study by Tegule, (2011). Consumption of fish contaminated with this organism leads to gastroenteritis manifested oftenly diarrhoea (Lopes da Silva *et al.*, 2010), the severity however depends on strain ingested. In spite of the presence of *E. coli* in fresh fish, the study showed that, traditional smoking process destroyed the organisms to acceptable levels (Gilbert *et al.*, 2000). The destruction was attributed to high sensitivity to heating during hot-smoking. Similar significant *E. coli* reductions were also reported in Cameroon (Ali *et al.*, 2011) and Sudan (Egbal *et al.*, 2013). While smoking eliminated *E. coli*, the study revealed very high mean microbial load for on shelf smoked fish from markets except for Nakulabye. This evidently indicated re-contamination with fecal matter, which was a pointer for poor handling conditions. Furthermore, the presence of similar *E. coli* numbers was also reported in Ghana (Arthur & Somuah, 2004). In view of these findings, on shelf smoked fish from markets in Uganda, presented a potential health hazard to consumers more so for those who don't reheat or cook smoked fish before consumption.

From the study, mean microbial counts for *S. aureus* in fresh fish with exception of Katosi samples, were higher than routinely accepted levels ($<10^5$ cfu/g) according to Lopes da Silva *et al.*, (2010). Presence of such high numbers indicated poor handling conditions among fish handlers. This is regard of the fact that majority (80%) of *S. aureus* organisms are harbored by humans as normal flora (FAO, 1992; Adebayo-Tayo *et al.*, 2012). Furthermore, while low numbers in raw fish are not a concern, very high *S. aureus* load can be hazardous (Adebayo-Tayo *et al.*, 2012), because *S. aureus* potentially elaborate an enterotoxin that is heat stable. In view of this prevention of higher numbers of *S. aureus* in food is a safer option, because the toxin can not be destroyed by heating. In contrast, the lower numbers reported in the previous study from Brazil (Lopez da Silva *et al.*, 2010), indicated that, *S. aureus* load can be reduced through improved sanitary conditions. Nonetheless, the heating process that provides internal temperature of above 65.5°C for 30 minutes readily destroys *S. aureus* (FAO, 1992; Tzouros & Arvanitoyannis, 2000). Accordingly, hot-smoking process eliminated *S. aureus* in all samples from Ggaba and Katosi, except for Kasenyi samples. Presence of higher load >3 log cfu/g than acceptable load of <2 cfu/g (ICMSF, 1986) in freshly smoked fish, necessitated proper re-heating of smoked fish before consumption but also shortening of its shelf-life to avoid further proliferation of the organisms. Furthermore, the presence of what is described as unsatisfactory (Gilbert *et al.*, 2000) numbers in samples from Kasenyi was probably attributed to higher initial numbers in fresh fish or re-contamination during smoking process. While hot-smoking process reduced *S. aureus* load to acceptable levels, the presence of very high numbers more than acceptable (<2 log cfu/g) (ICMSF, 1986) in samples obtained from the market, revealed a high possibility of recontamination. Based on study findings, it was also generally deduced that, smoked fish sold from Kampala markets were potentially hazardous to consumers.

Apart from bacteria, mycoflora including Yeasts and molds, have been used as indicators for microbiological quality of food or fish. This is so because, a number of mycoflora species are responsible for spoilage of different foods including fish, smoked or fresh. Whereas some species are useful in fermentation and ripening of some foods, others clearly cause spoilage of foods (Lorenzo *et al.*, 2018), hence, they can be used to indicate possibility of fish spoilage. According to the study, low fungal counts (4 log of cfu/g) were found in fresh samples, probably due to, high moisture content and may be fish habitat did not favor their proliferation. This is because, mycoflora require low water of activity for their proliferation and therefore, most species prefer growing on dried products (Ali *et al.*, 2011). Furthermore, the traditional smoking process significantly ($P<0.05$) reduced these organisms for Katosi and Kasenyi, except for the samples from Ggaba. The variation in mycofloral destruction was probably due to presence of different fungal species in fish. Regardless, in spite of the low numbers in fresh samples, generally heating did not eliminate all organisms from all the samples, in this case the role of spore formation known to considerably resist destruction by heating could not be underestimated. Nevertheless, while freshly smoked fish had low numbers, those from Bwaise still presented a potential quality concerns since their levels (4 log of cfu/g) were not within acceptable range. Therefore, on shelf smoked fish from Bwaise presented quality concerns and also a health hazard to consumers. The latter was particularly crucial considering their potential to elaborate mycotoxins elaboration. By and large, much as prolonged smoking with salting (5%) is encouraged for control of fungal growth (Omojowo *et al.*, 2010), periodic re-smoking pending fish sale could also be a viable alternative.

5. Conclusion

Fresh fish generally harbored unacceptably high microbial numbers for various indicator organisms. However, traditional smoking process reduced, and for some samples eliminated most microbes to acceptable levels, and this indicated considerable level of effectiveness. It is noteworthy that, while smoking process considerably destroyed the microbes, moisture content left in freshly smoked fish was still very high, and this indicated a relatively short shelf-life of traditionally smoked fish in Uganda. The presumed short shelf-life was deemed disadvantageous to the smoked fish consumers in remote areas, since accessibility before quality loss was not guaranteed. Furthermore, although smoking significantly reduced all microbes investigated, fish re-contamination post-smoking due to poor handling conditions, was found to offset the usefulness of the widely used traditional smoking method. In view of this, much as freshly smoked fish was of good microbiological quality and fit for consumption, the same could not be said for smoked fish from the markets. Therefore, for assured smoked fish quality in Uganda, we recommend that; traditional fish smoking in Uganda be improved through; proper food handling awareness and training, use of improved kilns, and improved hygiene before, during and after smoking. Additionally, re-smoking of smoked fish before sale, holding of smoked fish for shorter time before sale, regular fish inspection, and proper cooking of on shelf smoked fish before consumption, are highly recommended.

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