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Microbiological quality and safety assessment of sun dried *Rastrineobola argentea (Mukene)* sold at selected landing sites of Lake Victoria and Peri Urban Kampala City Markets

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Silver fish (*Rastrineobola argentea*) also locally known in Uganda as *Mukene* contributes significantly to Ugandan national economy and its value was estimated at $13 million US dollars in 2015. The fish is traditionally dried under direct sunshine on bare ground in unhygienic conditions, which expose it to dust and microbiological contamination. In this study, the microbial load of indicator and pathogenic organisms was determined in *Mukene* sold at selected landing sites of Lake Victoria and Kampala markets, Uganda. A total of 46 samples were collected randomly from landing sites and markets. The total aerobic counts, total coliforms, *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* were enumerated using standard microbiological methods. The findings showed that *Mukene* was of low microbial quality for total plate counts, total coliforms, *E. coli* and *S. aureus* counts with values ranging from 2.48-8.61 log cfu/g, 0.36-3.09 log MPN/g, 0.36-3.04 log MPN/g and 0.10-6.66 log cfu/g, respectively. Of all samples analyzed, 63% were positive for *Salmonella* species. As salmonellae and staphylococci are often implicated in incidences of food poisoning, this study suggests that consumption of sun dried *Mukene* sold at landing sites of Lake Victoria, Uganda, poses a public health concern. There is the need to improve on hygiene during processing, storage and distribution of *Mukene* in Uganda.

Key words: Silver fish, food safety, contamination.

INTRODUCTION

Silver fish (*Rastrineobola argentea*) also locally known in Uganda as *Mukene* is a silvery tiny fish with an average length of 5 cm and average weight of 15 g (LVFO, 2012). Based on the December 2015 Catch Assessment Surveys (CASs), the commercial fisheries on the part of Lake Victoria in Ugandan is currently dominated by the by three species namely; *Mukene* (65268.6 MT, 43%), Nile perch (*Lates niloticus*; 377,219.3 MT, 25%) and Nile

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Tilapia (*Oreochromis niloticus*; 13,278.2 MT, 9%). *Mukene* value was estimated at 13 million US dollars, which is a significant contribution to the Ugandan national economy (Nakiyende et al., 2016).

According to Masette and Kwetegyeka (2013), about 80% of *Mukene* was processed into animal feeds and only 20% was marketed for human consumption. Since 2009, there has been an increase in production of *Mukene* for human consumption as the price of other sources of animal protein has risen sharply. Today, more local consumers, who had previously attached a negative social attitude towards *Mukene*, have reverted to its consumption. The market is so large and lucrative that *Mukene* of questionable quality is also sold at a high price (Masette and Kwetegyeka, 2013).

In Uganda, the commonest method of preserving *Mukene* is by drying in the open sun on bare ground or gravel (Figure 2) of which such conditions expose the fish to physical and microbiological contamination. Similar conditions are reported in the research carried out on the shores of Lake Victoria, Kenya by Onyango et al. (2015). Determination of microbiological quality of such processed fishes from the market is important for safe guarding the consumer's health and hygiene (Sinduja et al., 2011). The quality of preserved fish is linked to the handling, processing and post processing procedures of the fish during which the fish is susceptible to microbial attack. Microorganisms of major concern in fish and its products include *Escherichia coli, Staphylococcus aureus, Salmonella* typhimurium*, Bacillus cereus, Shigella* spp*.* and *Clostridium botulinum* (Nunoo and Kombat, 2013). Food products contaminated with faecal matter pose a great risk to human health as they are more likely to contain human-specific pathogens. Indicator microorganisms in food microbiology have been used to predict the presence of potential risks associated with pathogenic microbes (Sifuna et al., 2008). The enteric bacteria include *E. coli* which is always considered to be of faecal origin (Sifuna et al., 2008).

In addition, *Salmonella* is a member of Enterobacteriaceae family, Gram-negative, facultative anaerobe, motile, with peritrichous flagella, non-spore forming rods that are responsible for causing salmonellosis. In humans, these pathogenic bacteria cause enteric fever (typhi or paratyphi) and acute gastroenteritis (Olgunoğlu, 2012). Finally, *S. aureus* is the most common food poisoning organism (Hennekinne et al., 2012) that produces enterotoxins. Staphylococcal enterotoxins are an important intra dietetic intoxication in the world (Kadariya et al., 2014). Contamination of dried *Mukene* by these organisms could occur through processing, storage, transportation and during sale at open air markets (Yusuf and Hamid, 2017; Geetha et al., 2014).

In Uganda, several strategies have been laid to minimize the level of pathogens in the sun-dried *Mukene*. Some of the strategies include regular inspection of *Mukene* processing facilities and sensitization of fishermen on good fishing practices. However, little has been done to assess microbiological quality and safety of the fish sold at different landing sites of Lake Victoria and City markets in Kampala. This study assessed the microbiological quality and safety of *Mukene* sold at landing sites of Lake Victoria and City markets in Kampala, Uganda.

MATERIALS AND METHODS

The study was carried out at three gazetted landing sites, where large quantities of *Mukene* catches are landed and sold on a daily basis during the harvesting season, and three retail markets in and around Kampala city where silver fish is sold in various packages on a daily basis. The landing sites included Kiyindi, Lambu and Maruba on Lake Victoria. Kiyindi landing site (Landing site 1) is located in Najja sub county, Buikwe District. It is 20 km from Lugazi town along Kiyindi ferry road. The silver fish is usually dried on raised racks and on old fish nets (Figure 1).

The Government of Uganda through the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) constructed good facilities used for storing large quantities of *Mukene* processed from this landing site. The stores handle about 50 tonnes of *Mukene* per day, during the peak season. The site has a population of about 40,000 people.

Lambu landing site (Landing site 2) is located in Masaka District, Bukoto East County with a population of about 8,000 people, on the western shores of Lake Victoria. It is 38 km from Masaka town along Bukakata road. Three types of fish are landed there namely Nile perch, tilapia and *Mukene*. Of the three, *Mukene* is the major catch for 80% of the boats. At this landing site, 80% of *Mukene* is dried on bare ground (Figure 2), 10% on old fish nets, and 10% on raised racks. During the peak season of January to April, the total harvest amounts to 110 tones (DFR-MAAIF, 2012). Maruba landing site (landing site 3) is located in Buhemba sub county Namayingo District. It has a population of about 2,500 people. It is 32 km from the District headquarters along Namayingo –Maruba road. About 50 boats out of 210 are normally used for fishing *Mukene* and during the peak season, about 3.6 tonnes of the fish are harvested (DFR-MAAIF, 2012). The Mukene catches are entirely dried on old fish nets (Figure 3). The three markets from which samples were taken are located around central region in Kampala City, Uganda. *Mukene* is usually sold in packages of kilograms in these markets.

Sample collection

Freshly dried samples of about 50-100 g were bought from artisanal processors drying *Mukene* at landing sites of Kiyindi, Lambu and Maruba. Other samples were randomly collected from traders at Kiyindi landing site. Older samples were collected from retail fish mongers in 3 markets in Kampala city. All samples for microbiological analysis were collected between June and August 2017.

Study design

The study was a cross-sectional survey to determine the microbial load of indicator and pathogenic organisms in *Mukene* sold at selected landing sites of L. Victoria and Kampala markets, Uganda.

Sample size determination

The sample size for the *Mukene* collected was determined using

Figure 1. Artisanal processors drying *Mukene* on raised racks at Kiyindi landing site.

Figure 2. Mukene drying on bare ground at Lambu landing site.

Figure 3. Women drying Mukene on old fish net on the ground at Maruba landing site.

the following formula adopted from

$$n = \frac{Z^2 P(1-P)}{d2}$$

Where n was the sample size, Z^2_{α}=1.96 and corresponding to 95% of statistical level of confidence, prevalence of *Salmonella* spp in sundried silver fish P=15% (Baniga et al., 2017). d^2= 10% precision, the maximum error that can be tolerated in the study:

$$n = \frac{1.90^2 \times 0.15(1-0.15)}{(0.1 \times 0.1)}$$

$$\mathrm{n} = \frac{0.4603}{0.01}$$

n=46

Sampling

Stratified sampling was employed when collecting samples whereby the landing site, supermarkets and retail markets constituted the sampling strata.

Media preparation

Standard plate count agar (PCA) CONDA CAT; 1056.00

A total of 23.5 g of the media was suspended in 1 L of distilled water. The medium was mixed well, dissolved by heating with frequent agitation and boiled for 1 min until complete dissolution. The medium was then dispensed into appropriate containers and sterilized in an autoclave (Model: TS-AJ) at 121°C for 15 min.

Brilliant green bile broth (BGB) CONDA CAT: 1172.00

Up to 40 g of the medium was suspended in 1 L of distilled water, mixed well and dissolved by heating with frequent agitation. It was then boiled for 1 min until complete dissolution and dispensed in 10 ml volumes into test tubes containing inverted Durham gas collecting tubes for gas detection. All media were sterilized in an autoclave at 121°C for 15 min.

Buffered peptone water (BPW) –Bio lab PBE20500

The medium (16 g) was added in 1 L of distilled water, heated gently to dissolve completely and distributed into 10 ml universal tubes. All tubes were then sterilized by autoclaving at 121°C for 15 min.

Brain heart infusion broth (BHI)

A total of 37 g of BHI was added to 1 L of distilled water, mixed well and distributed into 10 ml test tubes. The test tubes were then sterilized by autoclaving at 121°C for 15 min.

E. coli broth (EC)

Up to 37 g of the medium was dissolved in 1 L of distilled water and dispensed into final containers and sterilized by autoclaving at 121°C for 15 min.

Nutrient agar (NA)

Nutrient agar (28 g) was added in 1 L of distilled water and boiled to dissolve completely. The medium was then sterilized by autoclaving at 121°C for 15 min.

Triple sugar iron agar (TSI)

About 64.6 g of the medium were suspended into 1 L of distilled water, mixed well and dissolved by heating for 1 min with frequent agitation. The medium was dispensed into 10 ml, tubes sterilized in autoclave at 121°C for 15 min. It was allowed to cool in a slated position in order to obtain butts of 1.5-2.0 cm deep.

Data analysis

Data were analyzed using IBM SPSS package (version 23). Means were separated by One-way Analysis of Variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc test. Significance was defined at P<0.05. Results are presented as means and standard deviation.

RESULTS

The purpose of this study was to determine the microbiological quality and safety of sun dried *Mukene* (*R. argentea*) sold at selected landing sites of Lake Victoria and peri urban Kampala city markets. The results are displayed in Tables 1 and 2.

Table 1 shows the Total plate counts, Total coliform counts, *Escherichia coli* counts and *S. aureus* in *Mukene* sold at the selected landing sites and open retail markets. There were significant differences in the microbial counts among the sources for all the parameters studied (P< 0.05). Total plate counts ranged from 3.8-6.2 log cfu/g. They were the highest in landing site 3 and lowest in market 3. Overall, there were no differences in total coliforms between landing sites and markets. Total coliform counts ranged from 0.15-2.24 log MPN/g and differed significantly among the sources. Market 3 had the lowest counts, while all the other fish sources did not show any significant differences among themselves.

E. coli counts ranged from 0.09-2.06 log MPN/g and were significantly different among the fish sources. Market 3 had the lowest count, whereas market 1 had the highest count for *E. coli*. *S. aureus* ranged from 1.51-4.66 log cfu/g and it varied significantly among the fish sources. Market 3 had the lowest count and landing site 2 had the highest count for *S. aureus*. Among the landing sites, site 3 recorded the lowest count, while landing site 2 had the highest. There was no *Salmonella* detected in samples from market 3 and the detection was only for market 1. Focusing on landing sites, landing sites 1 and 3 had the highest detection levels of the organism, while landing site 2 had the least (Table 2).

Table 1. Total plate counts, total coliform counts, *E. coli* counts and *S. aureus* in *Mukene* sold at the selected landing sites and open retail markets.

Source of silver fish	Mean ± Standard Deviation			
	Total plate counts (log cfu/g)	Total coliform counts (log MPN/g)	*E. coli* counts (log MPN/g)	*S. aureus* (log cfu/g)
Market 1	5.0±1.3^{b}	1.83±1.03^{a}	2.06±1.03^{a}	1.83±1.49^{bc}
Market 2	5.3±0.8^{ab}	1.78±1.30^{a}	1.02±1.30^{ab}	4.15±1.68^{ab}
Market 3	3.8±0.8^{c}	0.15±0.24^{b}	0.09±0.17^{b}	1.51±1.30^{c}
Landing site 1	6.0±0.6^{ab}	2.24±1.31^{a}	1.96±0.86^{a}	3.91±2.30^{ab}
Landing site 2	5.7±1.1^{ab}	2.01±1.11^{a}	1.48±1.41^{ab}	4.66±1.30^{a}
Landing site 3	6.2±1.6^{a}	2.23±1.35^{a}	1.04±1.29^{ab}	3.09±1.85^{abc}

Values bearing different superscripts down the columns are significantly different.

Table 2. *Salmonella* spp. in *Mukene* sold at the landing sites and on retail markets.

Sample	Market 1	Market 2	Market 3	Landing site 1	Landing site 2	Landing site 3
1	Present	Present	Absent	Present	Present	Present
2	Present	Absent	Absent	Present	Present	Present
3	Present	Absent	Absent	Present	Present	Present
4	Present	Present	Absent	Present	Present	Present
5	Present	Present	Absent	Present	Absent	Absent
6	Absent	Present	Absent	Present	Absent	Present
7	Present	Present	Absent	Absent	Present	Present
8	Present	Present	-	-	Absent	-
9	-	-	-	-	Absent	-

Note the dashes in the table indicate no samples collected.

DISCUSSION

Overall, there was low microbiological quality and safety of the fish sold at selected landing sites of Lake Victoria compared to peri urban Kampala city markets. The findings showed that landing sites generally had higher Total Plate Counts averaging 5.75 log cfu/g among the indicator organisms. Among the pathogenic microorganisms, *S. aureus* had higher mean counts of 4.66 log cfu/g among the landing sites compared to the markets. Also, *Salmonella* spp. was found present in the most of the samples. These detection levels were above the locally acceptable standard limits for *Mukene* sold for consumption as recommended by the Uganda National Bureau of Standards (US_780, 2012). The presence of high levels of these pathogens may be attributed to poor hygiene practices and failure to employ hazard analysis critical control point (HACCP) along the production, processing and distribution chains. The results were similar with those of a related study conducted in Kenya that showed that there were feacal coliform contaminations in *Mukene* sold in the urban markets (Sifuna et al., 2008). Also, Baniga (2015) revealed higher higher mean bacterial counts of 6.7 log cfu/g in fresh fish.

The microbiological load was higher for the *S. aureus* and low for *E. coli* for the samples analyzed. The findings showed that *S. aureus* had a mean count of 4.66 log cfu/g. This was beyond the locally acceptable limit of 3.30 log cfu/g set by Uganda National Bureau of Standards. This may have been due to poor handling practices during processing at the landing sites. Fish at landing sites in Uganda is sometimes subjected to open sun drying on bare ground where domestic animals and wild birds excrete wastes on *Mukene* compared to the markets where such kinds of practices do not exist. A study by Amegovu et al. (2017) and Budiati et al. (2011) showed higher counts of *S. aureus* in fresh fish at the landing sites. This may have been due to high moisture content of fish at the landing site favoring microbial growth.

Generally, *Mukene* had a moderate *E. coli* load. The findings showed that *E. coli* had a high mean count of 1.8 log MPN/g in market 1 and a low mean count of 0.08 log MPN/g in market 3. The presence of *E. coli* in *Mukene* was an indication of the feacal contamination that had occurred in the fish sold from these markets. Similar findings were reported in a research done in Kumasi metropolis (Antwi-Agyei et al., 2017). The fish sampled

Table 3. Microbiological limits for *R. argentea (Mukene).*

S/N	Type of microorganisms	Maximum limit	Method of test
1	*Salmonella*	Absent in 25 g	ISO 6579
2	*Escherichia coli*	Absent in 1 g	ISO 7251
3	*Staphylococcus aureus*	3.3log cfu/g	ISO 6888
4	Total viable count	3.69 log cfu/g	ISO 7933
5	*Clostridium perfringens*	Absent	ISO 7937
6	*Listeria monocytogenes*	Absent	ISO11290-2
7	Yeast and Moulds	2 log cfu/g	ISO 2152-1
8	Coliforms	Absent in 100 g	ISO 4832

Source: DEAS 826:2014; Sifuna et al. (2008).

from the landing sites had mean counts of 1.7 log MPN/g. The acceptable local standard recommends no presence of any amount *E. coli* in the food products (Table 3).

Salmonella species were present in certain samples (Table 3). The presence of *Salmonella* spp. was an indicator of poor food preparation and handling practices such as cross contamination (Akabanda et al., 2017). Contamination of fish and fishery products with *Salmonella* has been reported by other researchers (Sinduja et al., 2011). Incidence of pathogens in the samples of fish on market may be attributed to external contamination and poor handling at ambient temperature. In the comparison with bacterial counts between markets and landing sites, landing sites had higher mean counts than the retail markets. Market 3 had lower bacterial counts and no *Salmonella* detected. This could possibly be due to the proper packaging of *Mukene* products by the suppliers. A similar study in Kenya found that there were low bacterial counts from the fish samples from commercially packaged silver cyprinid compared to the sun dried samples (Tieli et al., 2017).

Conclusion

This study revealed that *Mukene* sold around landing sites in Uganda need to be tested first for microbiological quality and safety. The microbiological load was higher for the *S. aureus* and lower for *E. coli* for the samples analyzed. The presence of *Salmonella* spp. may have been due to poor food preparation and handling practices such as cross contamination.

RECOMMENDATIONS

Based on these findings, there is the need to employ good hygiene practices (GHP) and hazard analysis critical control point (HACCP) along the production, processing and distribution chains in order to attain dried *Mukene* of high microbiological standard. Further research could focus on diagnosing the causes of such pathogens in *Mukene* being sold in all sorts of places.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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