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DEPARTMENT OF ZOOLOGY, ENTOMOLOGY AND FISHERIES SCIENCE

BACHELOR OF SCIENCE IN FISHERIES AND AQUACULTURE

THE MICROBIAL QUALITY OF TRADITIONALLY SMOKED NILE TILAPIA (*Oreochromis niloticus*)

BY

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF ZOOLOGY, ENTOMOLOGY AND FISHERIES SCIENCES OF THE COLLEGE OF NATURAL SCIENCES IN PATIALFULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A DEGREE OF BACHELORS OF SCIENCE IN FISHERIES AND AQUACULTURE OF MAKERERE UNIVERSITY

JULY, 2024

DECLARATION

DECLARATION

I KATUMBA HENRY declare that this work is true and original of my efforts and it has never been submitted to any Institution of higher learning for any academic award.

SIGNATURE……………………………… **DATE**…26/07/24……….

KATUMBA HENRY

APPROVAL

APPROVAL

This is to affirm that I have approved this dissertation for submission.

SIGNATURE.............................. DATE....28/7/2024

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Supervisor

DEDICATION

I dedicate this dissertation to my entire family especially my father Mr. Kityo Godfrey and my mother, Mrs. Kityo Gertrude Namagembe, for their unconditional support and encouragement. I am stunned by the overwhelming guidance and financial support from my parents. I also dedicate this dissertation to all fish processors and researchers who are devoted to enhancing the quality and quantity of smoked fish.

ACKNOWLEGEMENT

Completion of this dissertation cannot be attributed to my individual effort alone, but to a multitude of individuals and institutions. Therefore, I would like to express my sincere gratitude to the following;

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LIST OF ACRONYMS

^0C	Degrees Celsius
E. coli	*Escherichia Coli*
ml	Milliliters
ICMSF	International Commission on Microbiological Specifications for Foods
TPC	Total Plate Count
S.aureus	*Staphylococcus aureus*
Cfu/g	Colony forming units per gram
SD	Standard deviation
N	Sample size

ABSTRACT

Microbiological quality of traditionally smoked fish was assessed to ascertain the 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. In light of this, the status of spoilage, hygiene and pathogenic indicator organisms in fish (Nile tilapia) was investigated. A total of 40 randomly selected on shelf smoked fish from Nakasero and Owino markets were obtained for microbial analysis. Descriptive statistics was used to present the data, while Tukey's test was used to analyze the data. The study revealed that, the mean microbial load (log of cfu/g) of indicator organisms for on shelf smoked fish (Nile tilapia) market samples was above the acceptable levels for consumption. Furthermore, the mean microbial loads of indicator organisms differed significantly ($P<0.05$) between the two markets of Nakasero and Owino.

Additionally, mean microbial load (log of cfu/g) for on shelf smoked fish from markets varied from; 6.78 to 8.82 (Total Plate Count), 0 to 6.12 (*E. coli*), and 4.15 to 7.05 (*S. aureus*).

Generally, the microbial status of on shelf smoked fish (Nile tilapia) from Owino and Nakasero markets 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.

CHAPTER ONE

INTRODUCTION

1.1 Background

Fish is a highly perishable product due to its high water and protein content which are essential nutrients that aid proliferation of microbes that reduce its lifespan and greatly affect its shelf-life and consumer safety. Furthermore, its less amount of collagen makes it soft and susceptible to physical damage during handling thus the need to prolong its shelf life (Olaleye and Abegunde, 2015).

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 with most of the fish being consumed in the smoked form (Adeyeye *et al*., 2016).

Furthermore, fish smoking is the most preferred preservation method, because smoke not only gives special taste, color and aroma to food, but also enhances preservation due to the dehydrating, bactericidal and antioxidant properties of smoke (Adeyeye, 2019).

Typical smoking of fish is either cold (28–32°C) or hot (70–80°C). Cold smoking does not cook the flesh, coagulate the proteins, inactivate food spoilage enzymes or eliminate the food pathogens, and hence refrigerated storage is necessary until consumption (Alasalvar *et al*., 2011)

Hot smoking on the other hand, cooks flesh of the fish, the heat produced destroys bacteria resting on and inside the fish, de-activates the enzymes in the guts and flesh and also dries the fish (Adeyeye, 2019).

The fish industry in Africa is dominated by artisanal fishing and fish processors who operate on small-scale and it is for this reason that traditional smoking is the most prevalent method of smoking in developing countries since it is cheap as it requires low initial capital investment affordable by subsistence processors (Adeyeye and Oyewole, 2016).

Furthermore, fish smoking is preferred to other methods due to low costs of production, simple artisanal skills required, and high demand for smoked fish. Coincidentally, this method is the most widely used for preserving fish in Uganda, with Nile perch (*Lates niloticus*) and Tilapia (*Orechromis niloticus*) fish species, being the frequently smoked by artisanal processors (Abigaba *et al*., 2020).

With the ever growing population comes, the need to store and transport the fish from one place to another where it is needed and fish preservation becomes necessary in order to increase its shelf life and maintain its nutritional value, texture and flavor.

Therefore in order to meet the increasing demand from the ever growing population, increased levels of quality smoked fish should be maintained. Thus efficient smoking becomes a mandate to avoid deterioration in quality through ensuring critical and careful handling of fish is observed during smoking.

Salaudeen and Osibona (2018) suggested that the smoking method, the technique adopted and handling practices certainly influence both microbial safety and shelf-life of smoked fish.

In view of this, high bacterial 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 and Oyewole, 2016).

Additionally, considering reports of high spoilage and pathogenic microbial load in smoked fish from different countries, there is need to ascertain microbiological status of traditionally smoked fish from Uganda where information about the same, is largely scanty and inconsistent. Hence, this study will quantify the microbial load of smoked fish in selected markets as a tool for surveying the effectiveness of the handling conditions and a measure of the shelf life and safety of smoked fish consumed locally.

1.2 Statement of the problem

Smoked Nile tilapia is a delicacy to citizens of Uganda since it is readily available and cheap thus they consume it as their source of protein. However, during handling, storage and transportation different undocumented mechanisms are used and since 30% of landed fish are lost through microbial activity alone (Ghaly *et al.*, 2010), the microbial safety of smoked Nile tilapia becomes a concern.

This is as a result of exposure to microorganisms some of which are pathogenic to humans directing exposing consumers to health risks. Food poisoning and diarrhea are some of the health disorders that can arise from consumption of contaminated smoked Nile tilapia. Such challenges can only be overcome by buffering up the inconsistent and scanty available information about the microbial safety of traditionally smoked Nile tilapia.

Therefore, studies are required to bridge the gap between bacterial spoilage and deterioration of smoked fish thus this study will quantify the microbial load of smoked Nile tilapia on market stalls to create awareness to the players along the value chain to improve the handling practices of smoked fish.

1.3 Objectives

1.3.1 General Objective

To assess the consumer safety of traditionally smoked *Oreochromis niloticus* (Nile tilapia) on market stalls in order to create awareness to improve handling practices.

1.3.2 Specific Objectives

I. To determine the Total Plate Count (TPC) of traditionally smoked Nile tilapia of selected markets.

II. To determine the load of *Esherichia coli* and *Staphylococcus aureus* of traditionally smoked Nile tilapia.

1.4 Null Hypotheses

I. There is no significant difference in the load of the total plate count of smoked Nile tilapia collected from selected markets in Uganda.
II. There is no significant difference in the load of *Esherichia coli* and *Staphylococcus aureus* of smoked Nile tilapia collected from different selected markets in Uganda.

1.5 Significance of the study

This study will determine the bacteriological quality of traditionally smoked *Oreochromis niloticus* from two selected markets, Owino and Nakasero in Kampala district. This will be achieved through quantifying the load of *E. coli* and *S. aureus* thereby informing the local consumers about the safety aspects of ingesting such fish. The empirical data from this study will assist policy makers to regulate and design appropriate standard handling practices to ensure that microbial contamination of smoked fish is well managed.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nile Tilapia (*Oreochromis niloticus*)

It is one of the most important aquaculture species and currently the third-most cultivated fish in aquaculture, after grass carp and silver carp (FAO, 2020a). In Africa, including in its native habitat and introduced areas, it is often the most important food-fish both in inland capture fisheries and aquaculture production (FAO, 2020a, b).

Aquaculture production of Nile tilapia contributes more than 80% of tilapia production globally (FAO, 2018). Even though the native genetic resources for Nile tilapia are from Africa, most of the top Nile tilapia aquaculture-producing countries are from Asia, with the exception of Egypt. From sub-Saharan African countries, Ghana, Uganda, Nigeria, Zambia and Kenya are significant producers from aquaculture (Adeleke *et al*., 2021).

The main reasons for the low production in sub-Saharan Africa include shortage of trained manpower, limited access to land, water and capital, lack of clear policy, low tradition of fish consumption, shortage of good quality brood stock, and lack of affordable locally produced fish feed (El-Sayed, 2017; Chan *et al*., 2019; Adeleke *et al*., 2021).

Thus due to *Oreochromis niloticus* being highly resistant to most adverse conditions like temperature, climate and diseases in addition to its fast adaptability to new environment (Geletu and Zhao, 2023) makes it readily available in Uganda and cheap for most artisanal fish processors who dominate the fishery sector in the country.

2.2 Traditional smoking in Uganda

Smoking, one of the oldest preservation methods, combines the effects of salting, drying, heating and smoking. Smoked fish is one that has been cured by smoking that is cooked, dried with a special taste and aroma added to it. (Adeyeye, 2019). The biggest percentage of fish catch in the developing countries is smoke-dried (Adeyeye *et al*., 2016).

Uganda in particular is among the countries whose local communities largely practice fish smoking more than other preservation methods using rectangular brick/mud kilns, Cut-out oil drum, Chorkor kiln and underground kilns (Abigaba *et al*., 2020).

Abigaba et al., (2020) identified fish smoking to be involved with transportation, reception and fish preparations like degutting, de-scaling, washing, salting, drying, loading, covering, lighting the fire in the kiln, un-hanging, packaging and storage of the fish.

In the light of this, irrespective of the inadequate hygienic handling practices during transportation, preparation, smoking, storage and marketing of fish, the smoking flow diagram followed in Uganda is similar to the generic smoking procedure used in other countries. This indicates potential for fish quality improvement once proper measures are enforced.

2.3 Pathogenic contamination of smoked fish.

Consumption of smoked fish is often associated with human disease especially when undercooked fish is ingested. The presence of different bacteria species including human pathogenic strains in smoked fish can be linked to direct contact with a contaminated environment, improper post-harvest handling and transportation (Novoslavskjj *et al*., 2015). Some of the bacteria contaminating smoked fish include; *Aeromonas spp, Clostridium perfringens, Escherichia coli, Listeria spp, Salmonella spp, Staphylococcus aureus* and *Vibrio cholerae* (Al Shabeeb *et al*., 2016).

In the light of this, *Escherichia coli* and *Staphylococcus aureus* have been reported in smoked Oreochromis Niloticus (Jega *et al*., 2019). Furthermore, *Staphylococcus aureus*, Coliforms, *Escherichia coli* have been reported in traditionally smoked *Oreochromis niloticus* in significant numbers higher than recommended (Abigaba *et al.,* 2021).

Staphylococcus aureus (S. aureus) is a Gram-positive spherically shaped bacterium, a member of the Bacillota, and is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen. It is not an inherent micro biota in fish but rather of handlers' origin through post-harvest contamination during processing (Murugadas *et al*., 2019). A ubiquitous organism that is mainly harbored by animals and most especially the human nose, throat and skin (Taylor and Unakal, 2022). Consumption of smoked fish contaminated with *Staphylococcus aureus* results in food poisoning that causes vomiting, diarrhea, and abdominal cramps within 2-6 hours from the time of ingestion (Artfatahery *et al*., 2015).

Escherichia coli (E. coli) is a gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms. *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for three days, but its numbers decline slowly afterwards (Smith and Framatico, 2017). *E. coli* pathogenic strains are divided into diarrhea inducing strains and the intestinal residing strains which may induce a watery diarrhea leading to dehydration. However the Shiga-toxin producing *E. coli* cause severe diarrhea or hemorrhagic colitis which may result in death (Smith and Framatico, 2017). Transmission to humans is through consumption of undercooked food products (Velhner *et al*., 2020), drinking contaminated water and from person to person through the hands to mouth routes (WHO, 2018).

2.4 Sources of pathogenic contamination in smoked fish

Smoked fish gets contaminated once exposed to the environment that is during transportation to the market, due to improper handling get contaminated with microbes. Sulav (2015) suggested that poor storage conditions allow pathogens to contaminate the fish.

Furthermore (Abigaba *et al*., 2020) enlightened that fresh fish was mostly (54.8%) transported without ice while directly placed on the floor of canoes, 26.9% packed it in sacks without ice; while 14.4% reported transportation under ice in a container. Only 3.8% reported transportation in ice on boat floor.

Contamination may also occur from processing instruments, fish processing table, markets stales and handlers. (Islam *et al*., 2021).

2.5 Acceptable limits safe for human consumption

The International Commission on Microbiological Specifications for Foods (ICMSF) is a body responsible for providing scientific concepts that when adopted by government agencies and industry reduce the incidence of microbiological foodborne illness and food spoilage worldwide and facilitate global trade.

The set standards recognized by the world health organization as safe for human consumption according to ICMSF are summarized in the Table 1.

Table 1: The set standards recognized by the world health organization as safe for human consumption

Microbial Parameter	Acceptable Limits	Source
Total plate count (TPC)	<7logcfu/g	(ICMSF, 1986)
Esherichia coli count	<2logcfu/g	(ICMSF, 1986)
S. aureus count	<2logcfu/g	(ICMSF, 1986)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study will be conducted at two markets i.e., Owino and Nakasero selected on the basis of market significance and consumer base in Kampala district. The two markets have different set ups and arrangements which aroused my curiosity for selection.

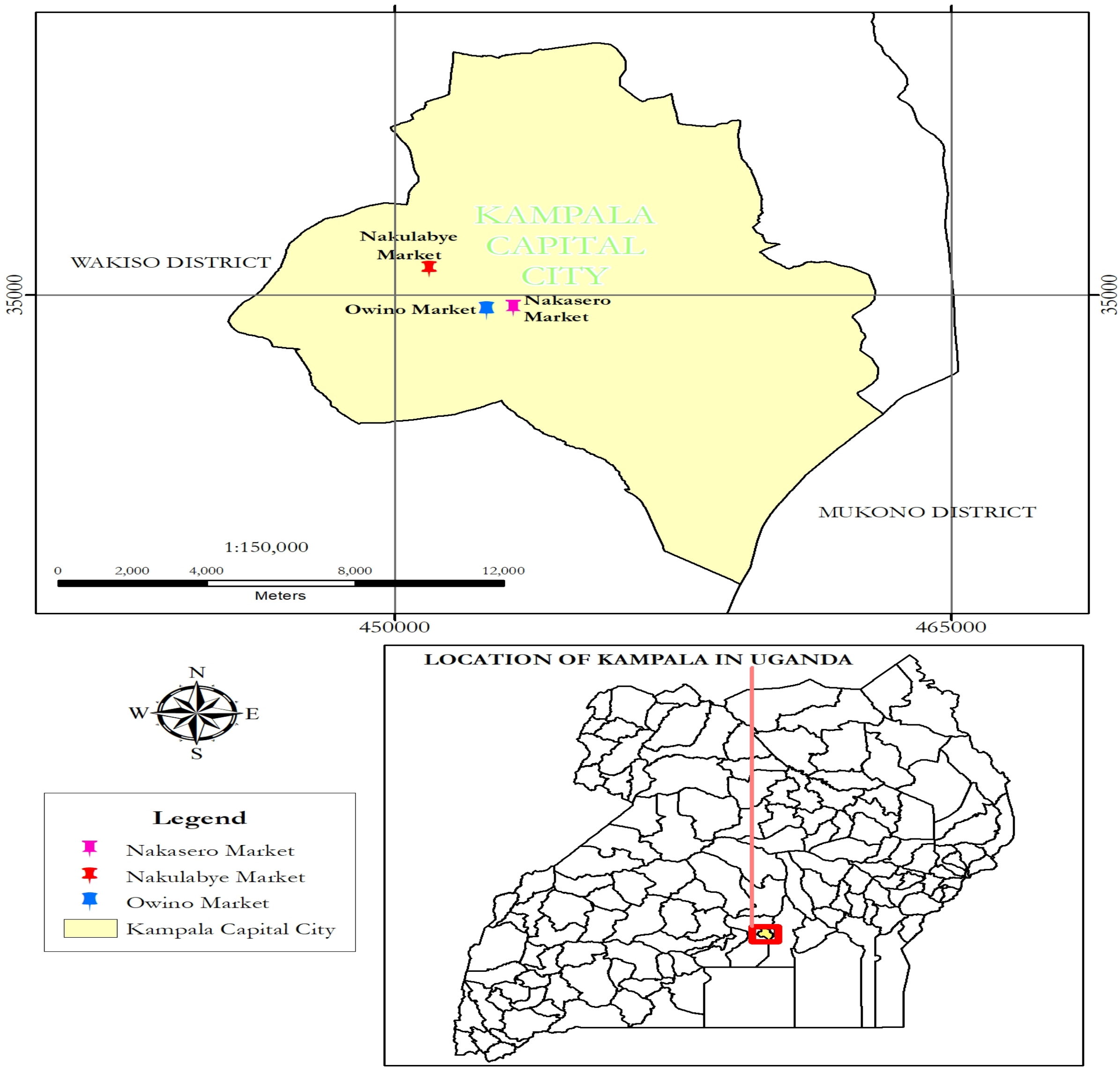


Figure 1: Location of Nakasero market and Owino market

3.2 Study design

A study design is presented in figure 2.

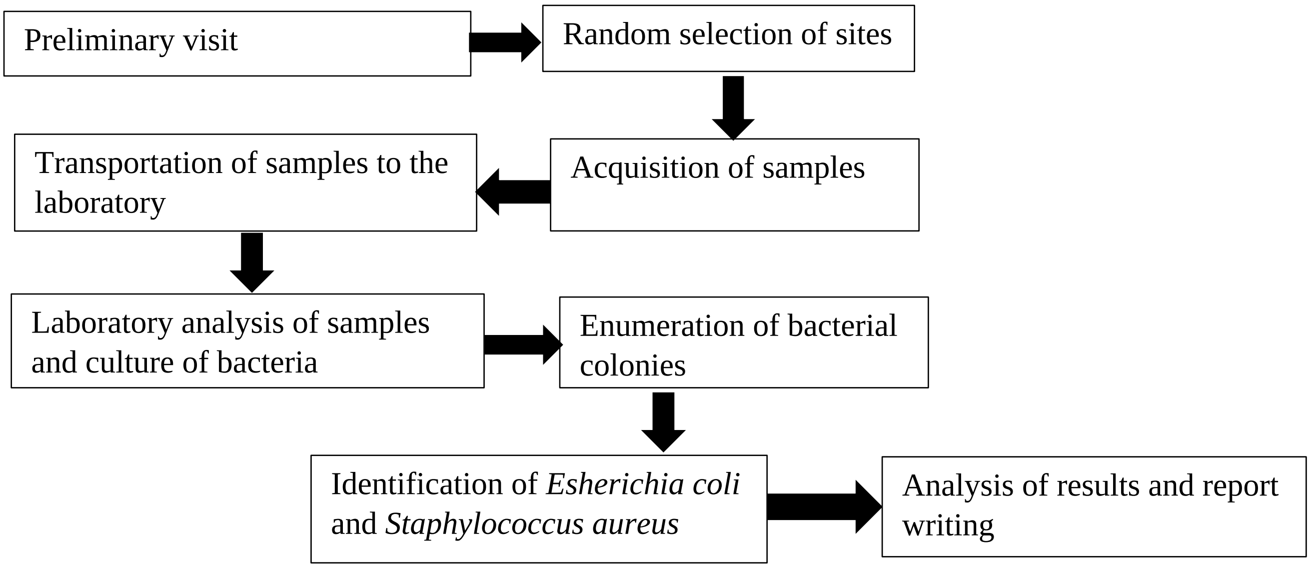


Figure 2: Study design

3.3 Sample collection

ICMSF sampling plan (1986) was used and a total of twenty smoked fish samples (N=20) were randomly selected from the top, bottom and sides on market stalls in sterile bags put on ice and transported to Central Biotechnology laboratory, college of Agricultural sciences Makerere University for analysis.

3.4 Data collection

3.4.1 Preparation of equipment and culture media

The working area was disinfected using 70% ethanol. All working equipment (Petri dishes, beakers, test tubes, conical flasks) were washed and sterilized in an autoclave at 121°C for 15 minutes.

Culture Media was prepared according to manufacturer's specifications and sterilized by autoclaving at 121°C at 15 psi pressure for 15 minutes and sterility of the media was checked by incubating at 37°C for 24 hours.

Distilled water was used for serial dilution, 10ml of each of the original samples with a dilution up to 10^{-5}. Serial dilution refers to series of sequential dilutions used to reduce a dense culture of cells to a more usable concentration.

3.4.2 Sample preparation

While ensuring asepsis, fish samples were removed from sterile bags and portions removed from the skin and muscle and blended using a pestle and mortar. Approximately 25g of the blended fish samples was weighed and added to 225 ml of Butterfield's buffer in sterile stomacher bags for homogenization in a stomacher at 12000 revolutions/minute for 1-2 minutes. Serial dilutions of 1 ml of homogenate were done for all samples up to 10^{-6}.

3.4.3 Bacteriological Analysis

Total Plate Count of the smoked fish samples was determined by pour plate method using nutrient agar. This was done by inoculating 1ml of the sixth serial dilution onto the sterile petri dish and the melted nutrient agar was then poured onto the petri dish, gently tilted to allow for uniform mixing. After solidification, the Petri dishes were then inverted and incubated at 37°C for 48 hours.

3.4.3.1 Isolation and identification of *E. coli and S. aureus*

S. aureus and *E. coli* were enumerated using surface spread method on Baired Packer mixed with egg yolk tellurite and Chromogenic Coliform Agar respectively. After 48 hours of incubation of the plates at 37°C.

This was done by mixing the sample potentially containing *E. coli* and *S. aureus* with Butterfield's buffer to create a range of dilution levels to ensure that the bacterial concentration becomes progressively lower. Subsequently Chromogenic Coliform Agar for *E. coli* and Baired Packer mixed with egg yolk tellurite for *S. aureus* were melted and poured into sterile petri dishes, ensuring a uniform distribution of the agar. Then 0.1ml of the inoculum from the serial dilution of 10^{-5} was spread onto the solidified agar. The Petri dishes were then inverted and incubated at 37°C for 48 hours.

Characterization and identification of isolated organisms was based on the appearance of the colonies by the color and shape.

E. coli colonies were observed to have a glossy purple appearance with a convex shape and several overlapping colonies while *S. aureus* colonies appeared as round shaped gray colored irregular clusters on the agar surface

3.4.3.2 Estimation of colony forming units per gram (cfu/g)

Following incubation, the plates were carefully examined, and the distinct colonies were counted. By considering the dilution factor, volume of the homogenate, weight measured to prepare the homogenate and the number of colonies counted, the original bacterial concentration in the sample was calculated and expressed as colony-forming units (CFUs) per gram using the formula below;

$$CFU/g = \frac{colonies\ counted \times 225ml \times dilution\ factor}{volume\ plated\ \times 25g}$$

3.5 Data Analysis

Microbiological data from laboratory was summarized using excel 2007 software using descriptive statistics in form of means and standard deviation (SD).

The Shapiro Wilk test was used to test for normality of the data. Tukey’s t-test was then used to assess the statistical differences between mean microbial loads of smoked fish of Nakasero market from that of Owino market at 5% level of significance (P=0.05).

CHAPTER FOUR

RESULTS

4.1 Microbial status of the smoked fish samples

Staphylococcus aureus and *E. coli* were present in all the examined smoked fish samples (N=20) except for two Owino smoked fish samples that were negative for *E. coli*. Furthermore, the log transformed values of colony forming units per gram (cfu/g) were summarized using descriptive statistics as presented in Table 2.

Table 2: Microbial load of smoked fish from the markets

Market	Statistic	Total Plate Count (log of cfu/g)	*S.aureus* Count (log of cfu/g)	*E.coli* Count (log of cfu/g)
Nakasero	Minimum	7.30	5.22	4.00
	Median	8.06	6.17	4.57
	Maximum	8.82	7.05	6.12
	Mean and SD	8.05±0.41	6.11±0.57	4.66±0.52
Owino	Minimum	6.78	4.15	0.00
	Median	7.40	5.37	3.44
	Maximum	8.02	6.80	4.18
	Mean and SD	7.44±0.33	5.36±0.49	3.17±1.11

4.1.1 Total Plate Count load of fish samples

A total of twenty fish samples (N=20) was analyzed for the TPC load of smoked Nile tilapia from Owino market and Nakasero market.

The study found that the TPC load (log cfu/g) of fish samples from Owino market ranged from 6.78-8.02 while that of Nakasero market ranged from 7.30-8.82. Furthermore, the median TPC load (8.06 log cfu/g) of fish samples from Nakasero market was higher than the median TPC load (7.4 log cfu/g) for fish samples from Owino market as illustrated in Figure 3.

Additionally, for all fish samples from Nakasero market the Total Plate Count load was above the recommended level (7 log cfu/g). However, for the case of Owino market, only two fish samples were below the recommended level with TPC loads of (6.78 and 6.83) log cfu/g. This is indicated by the lower whisker of the boxplot for Owino market (Figure 3) that goes below the line indicating the recommended level.

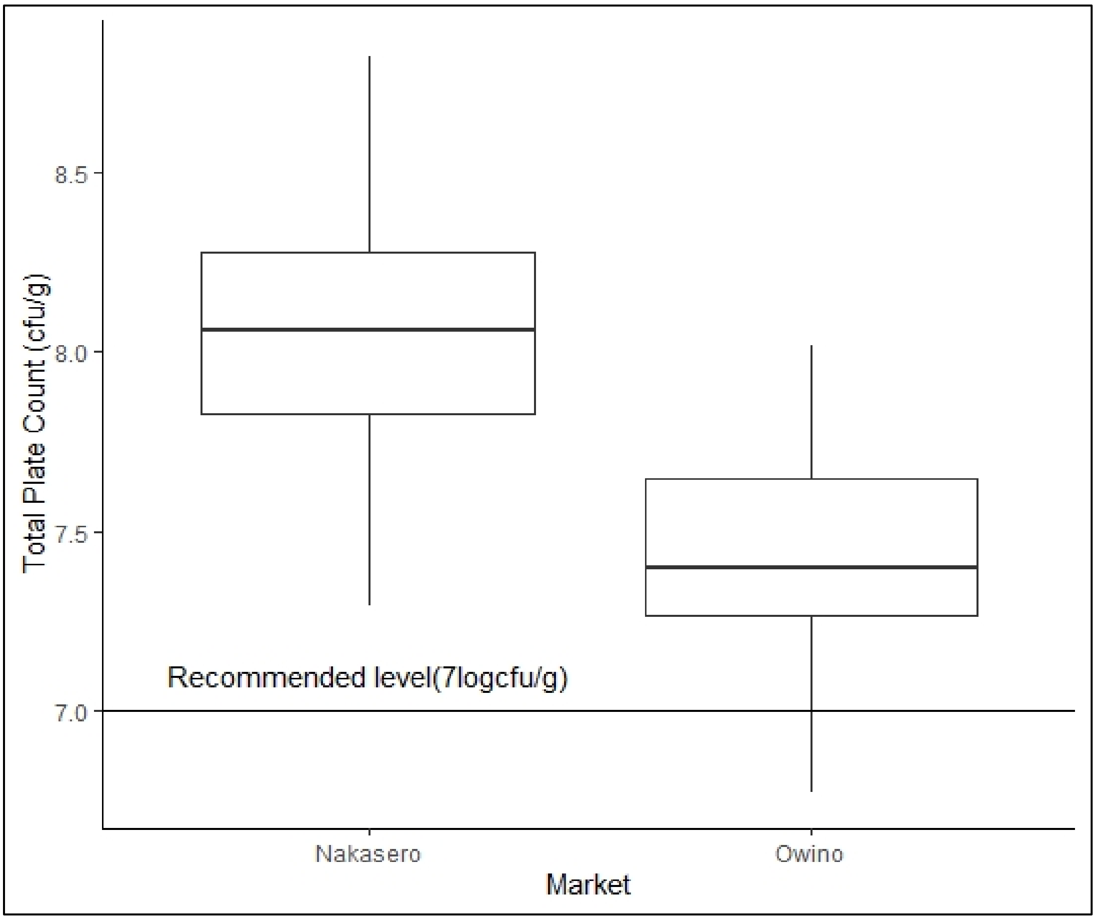
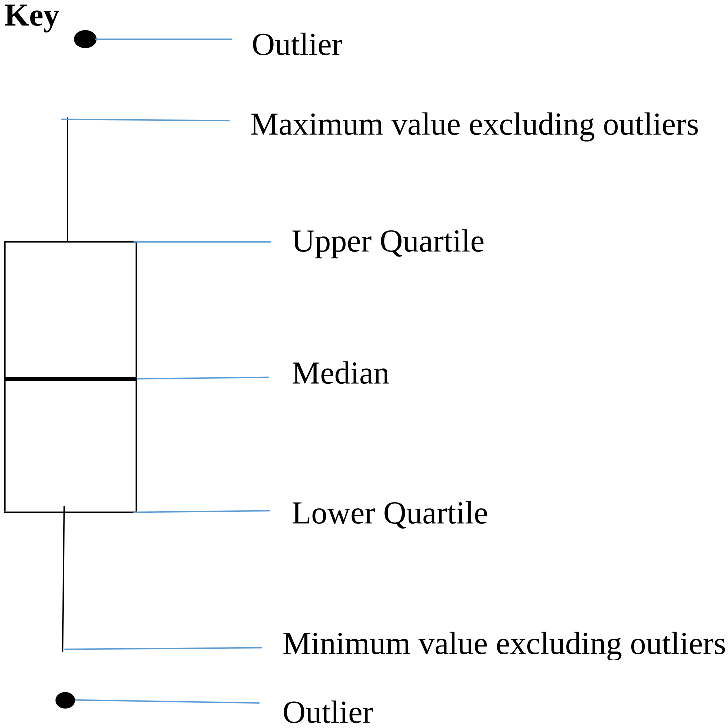


Figure 3: TPC load of fish samples from Owino and Nakasero markets

Key



4.1.2 *E. coli* load of the fish samples

A total of twenty fish samples (N=20) was examined for the *E. coli* load of smoked Nile tilapia from Owino market and Nakasero market.

The study found that the *E. coli* load (log cfu/g) of fish samples from Owino market ranged from 0-4.18 while that of Nakasero market ranged from 4.00-6.12. Furthermore, the median *E. coli* load (4.57 log cfu/g) of fish samples from Nakasero market was higher than the median *E. coli* load (3.44 log cfu/g) for fish samples from Owino market indicating a significant variation in the *E. coli* loads of fish samples between the two markets as seen in Figure 4.

Additionally, for all fish samples from Nakasero market the *E. coli* load was above the recommended level (2 log cfu/g) while for Owino market only two fish samples did not have *E. coli* indicated as a black dot in Figure 4.

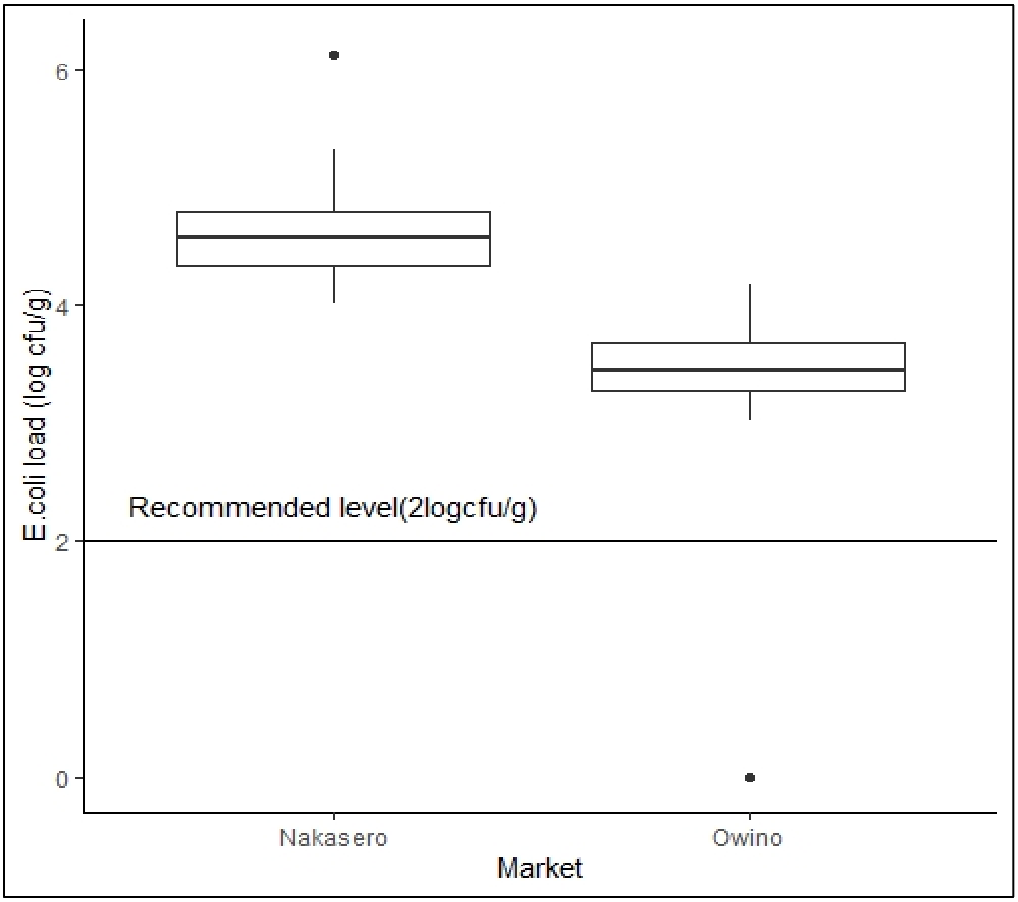


Figure 4: *E. coli* load of fish samples from Owino and Nakasero markets.

4.1.3 *S. aureus* load of the fish samples

A total of twenty fish samples (N=20) was examined for the *S. aureus* load of smoked Nile tilapia from Owino market and Nakasero market.

The study found that the *S. aureus* load (log cfu/g) of fish samples from Owino market ranged from 5.22-7.05 while that of Nakasero market ranged from 4.15-6.80. Furthermore, the median *S. aureus* load (6.17 log cfu/g) of fish samples from Nakasero market was higher than the median *S. aureus* load (5.37 log cfu/g) for fish samples from Owino market indicating a significant variation in the *S. aureus* loads of fish samples between the two markets as seen in Figure 5.

Additionally, for all fish samples from both Nakasero market and Owino market the *S. aureus* load was above the recommended level (2 log cfu/g) illustrated in Figure 5.

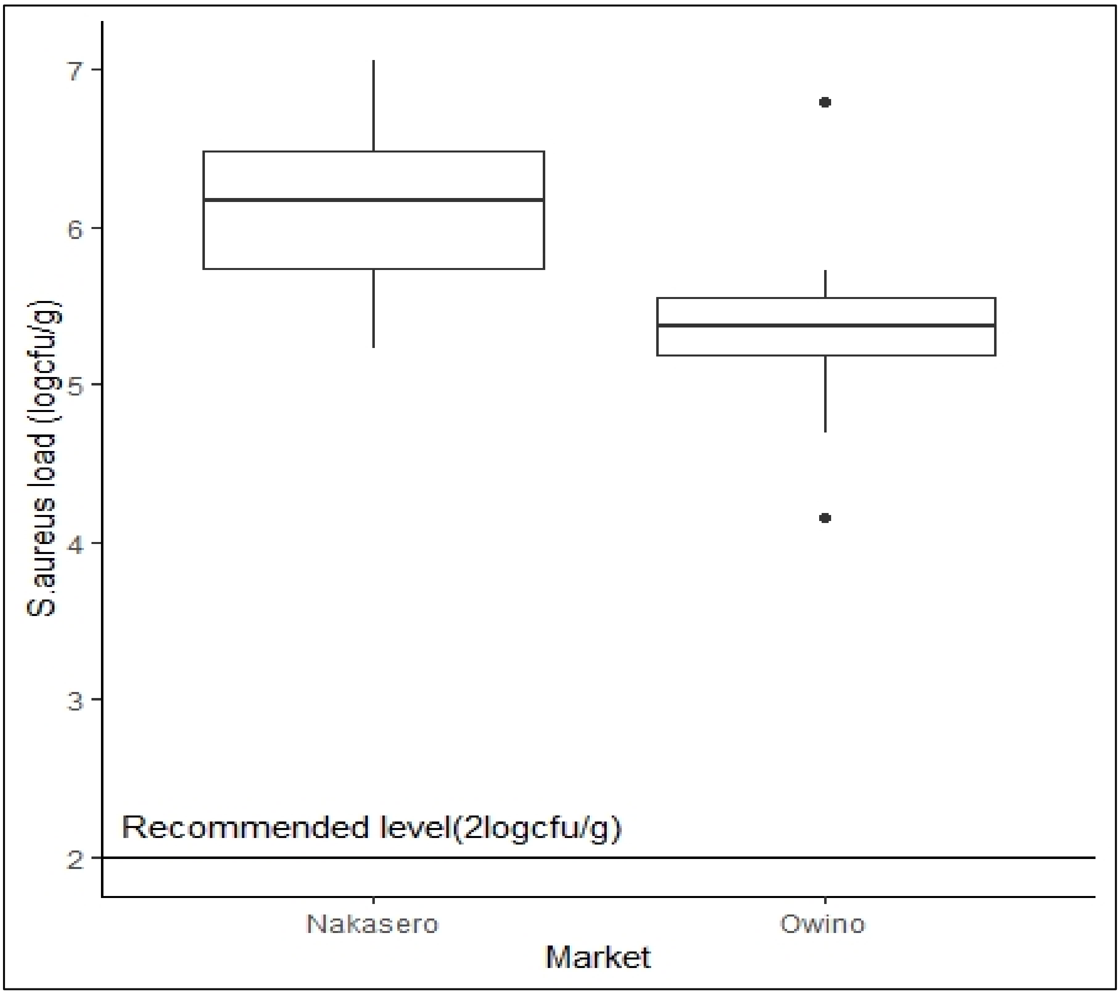


Figure 5: *S. aureus* load of fish samples from Owino and Nakasero markets

4.2 Differences in microbial load of the fish samples

Both *Staphylococcus aureus* and *E. coli* log transformed values were subjected to Shapiro Wilk normality test at P=0.05.

The means of the samples from the two markets were then subjected to an unpaired t test which indicated a significant difference between the means ($p<0.05$). The test statistics of the statistical tests are summarized in (Appendix 1).

4.3 Mean microbial load of smoked Nile tilapia

From the study findings (Table 4), samples from Nakasero had a higher mean microbial load than samples from Owino for all indicator organisms, all of which were above acceptable limits. The mean microbial counts (log of cfu/g) and corresponding standard deviations (SD) for smoked fish samples from both Nakasero and Owino markets are summarized in (Appendix 2).

CHAPTER FIVE

DISCUSSION

From the study, both spoilage and pathogenic indicator organisms were isolated from the smoked Nile tilapia samples which included; *S. aureus, E. coli* 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 total plate count (TPC) method (Diez-Gonzalez, 2014). Hence, the TPC was done to obtain a rough indication of microbiological quality of on-shelf smoked Nile tilapia from Nakasero and Owino markets.

Furthermore, the acceptable TPC load in smoked fish does not exceed 7 log of cfu/g (ICMSF, 1986), but from this study, all examined samples of smoked fish (N=20) obtained from the markets had aerobic mesophilic bacterial load above this value. This indicated poor handling conditions that is poor personal hygiene, poor sanitation of the storage facilities as well as water and equipment contamination which requires urgent attention from fish handlers and food quality agencies.

In the view of this, similar numbers of TPC load were reported in Uganda (Abigaba *et al.*, 2021; Abigaba *et al.*, 2020). However, the study findings contradict from (Abolagba *et al.*, 2011) who reported less numbers in Nigerian markets. This was probably due to the differences in smoking technology and fish handling as well as the initial microbial load in fresh samples before smoking.

Additionally, the differences in levels of TPC load between the two markets revealed by the study, could have been attributed to the varying levels of water contamination from different parts of the markets and the general poor handling by fish handlers as well as differences in market structure and organization. This is affirmed by (Abigaba *et al.*, 2021) who reported significant differences in the TPC load of smoked fish samples from markets of Bwaise and Nakulabye and which they attributed to differences in hygiene, poor handling and sanitation conditions of the markets.

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 the view of this, the presence of *E. coli* possibly indicates potentially more harmful microorganisms that are harder to detect due to lower numbers or specific environmental requirements.

Furthermore, Stec et al (2022) reported that the indicator role is particularly significant in monitoring water quality, as the detection of *E. coli* can prompt further investigation into potential sources of contamination and initiate appropriate corrective actions. Therefore, monitoring for *E. coli* in smoked fish can provide an early warning system for potential health risks and help to ensure that safety protocols are in place to prevent the transmission of pathogenic microorganisms to consumers.

In this study, presence of *E. coli* was investigated as measure for sanitary and health hazard monitoring. In spite of the fact that smoking eliminates *E. coli*, the study revealed very high mean microbial load more than acceptable limits (<2 log of cfu/g) (ICMSF, 1986), for on shelf smoked fish from both markets.

This evidently indicated re-contamination with fecal matter, which was a pointer for poor handling conditions. *E. coli* is indicative of health and safety risk because of the gastrointestinal tract origin (Roozbahani *et al.*, 2013). The bacteria indicate the high possibility of fecal contamination especially through water (Stec *et al.,* 2022), used in cleaning of the storage facilities.

The difference in *E. coli* load could be attributed to the variation in hygiene and sanitation of the storage facilities, location of market and protection of the on shelf smoked fish on the market from environmental contamination. In the light of this, studies have reported increased risk of contamination of plain tap water due to pipe leakage along the channel of distribution before reaching the users (Roozbahani *et al.,* 2013).

Water is known to be a vehicle for transmission of enteric pathogens usually contaminated by fecal materials due to poor disposal of human excreta (Nummer *et al.*,2015). In the same way, water contamination could have been due to use of untreated spring water sources in Kampala which are exposed to environmental contamination (Barakat *et al.,* 2018).

Staphylococcus aureus is not an inherent micro biota in fish but rather of handlers' origin through post-harvest contamination during processing (Murugadas *et al.*, 2019). It is a ubiquitous organism that is mainly harbored by animals and most especially the human nose, throat and skin (Taylor and Unakal, 2022). Therefore, in this study, presence of *S. aureus* was investigated as measure for hygiene, handling conditions and health hazard monitoring.

Whilst hot-smoking reduces *S. aureus* load to acceptable levels, the mean *S. aureus* counts (5.36 and 6.11) log of cfu/g for Owino and Nakasero respectively were more than acceptable limits (<2log of cfu/g) (ICMSF, 1986). Furthermore, the presence of similar *S. aureus* numbers was also reported in Uganda (Abigaba *et al.*, 2021).

Presence of such high numbers indicated poor handling conditions among fish handlers. This is in regard to the fact that majority (80%) of *S. aureus* organisms are harbored by humans as micro flora (FAO, 1992; Adebayo-Tayo *et al.*, 2012), thus the differences in the *S. aureus* load of fish samples was due to the variation in the handling conditions and personal hygiene of the fish handlers of the markets (Nakasero and Owino).

Furthermore, while low numbers in 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 thus prevention of higher numbers of *S. aureus* in food is a safer option, because the toxin cannot be destroyed by heating.

In view of these findings, on shelf smoked fish from Nakasero market and Owino market in Kampala, presented a potential health hazard to consumers more so for those who don’t reheat or under-cook smoked fish before consumption.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Generally, the microbial status of on shelf smoked fish (Nile tilapia) from Owino and Nakasero markets indicated poor fish handling, poor hygiene and sanitation of storage facilities, as they harbored unacceptably high microbial numbers for the indicator organisms (*E.coli* and *S. aureus)*.

The mean microbial load (log of cfu/g) for on shelf smoked fish from markets varied from; 6.78 to 8.82 (Total Plate Count), 0 to 6.12 (*E. coli*), and 4.15 to 7.05 (*S. aureus*).

In view of this, the microbiological quality of on-shelf smoked Nile tilapia from Nakasero and Owino markets is risky for consumption.

6.2 Recommendations

Therefore, for improved quality of on-shelf smoked fish, the study recommends the following; Proper food handling awareness and training, improvement of the market infrastructures, and improvement in hygienic conditions 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, should be put into practice.

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APPENDICES

Appendix 1: Statistical analytical results of microbial load of smoked fish

Parameter	**Test for Normality**		**Testing for significance**	
	Shapiro Wilk test		t test for unmatched pairs	
	W statistic	p-value	t statistic	p-value
Total Plate Count	0.98438	0.9775	-5.2086	7.749e-06
E.coli Count	0.9589	0.5221	-4.4085	8.487e-05
S.aureus Count	0.93345	0.1799	-5.4641	1.129e-05

Appendix 2: Mean microbial load of smoked Nile tilapia

Microbial Parameter	Owino market (log of cfu/g)	Nakasero market (log of cfu/g)	Recommended (log of cfu/g)
Total Plate Count	$7.44 \pm 0.33^{a*}$	$8.05 \pm 0.41^{b*}$	<7
E.coli Count	$3.17 \pm 1.11^{a*}$	$4.66 \pm 0.52^{b*}$	<2
S.aureus Count	$5.36 \pm 0.49^{a*}$	$6.11 \pm 0.57^{b*}$	<2

Key: Mean values with different superscripts (letters) in the same row, are significantly different, (P<0.05).

Values with symbol * are above recommended limits.

Appendix 3: A-Measuring 225ml of Butterfield's Buffer, B- Weighing 25g of fish sample to be used in preparation of the homogenate

Appendix 4: A-Melting of Baired Packer Agar, B-Preparation of nutrient Agar

Appendix 5: Sterilization of equipment and working environment

Appendix 6: Isolated indicator organisms; A- *E. coli*, B- *S. aureus* and C- Total Plate Count