MICROBIOLOGICAL AND CHEMICAL QUALITY OF *KAYABO* A SALTED SUN-DRIED NILE PERCH *Lates niloticus* FROM LAKE VICTORIA, TANZANIA

NURU EDGAR MWASULAMA

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF FOOD TECHNOLOGY, NUTRITION AND CONSUMER SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD QUALITY AND SAFETY ASSURANCE OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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EXTENDED ABSTRACT

Fish processing companies in Tanzania are basically for the export market with strict control and monitoring of the food safety hazards. The major processors of fish for the domestic market are small-scale processors often with limited knowledge on proper handling of fish, inadequate hygienic facilities, lack of adequate raw materials which compels them to purchase rejects (undersize, poor quality fish and fish frames) from fish processing companies. The use of rejects and poor quality raw materials associated with inadequate handling and processing conditions could result into food safety problems and endanger the public health.

This study was conducted to assess the handling practices as well as microbiological and chemical quality of salted sun-dried *Lates niloticus* (*Kayabo*) from Lake Victoria, Tanzania. Structured observation checklist was used to collect information from thirty *Kayabo* processors on the product handling practices along the processing chain. Samples were collected from processors from two Municipalities of Mwanza City namely Nyamagana (Kanyama) and Ilemela (Mwaloni). Analytical samples for microbiological analyses were prepared according to Andrew and Hammac (US - FDA BAM, 2001). Enumeration of total viable counts (TVC), total coliforms, *E. coli*, *S. aureus* were respectively performed according to ISO 4833:2003, ISO 4831:2006, ISO 7251: 2005, ISO 6888 – 3: 2003, whereas detection of *L. monocytogenes* was done according to ISO 11290-1:1996/Amd 1: 2004 methods. The total volatile basic nitrogen (TVB-N) of *Kayabo* was extracted following the European official method (EC) 2074/2005. To assay nitrogen specifically generated by the trimethylamine (TMA), distillation method was applied in presence of formaldehyde. Moisture content was determined by AOAC method 985.14 (AOAC, 1995).

Hierarchical cluster analysis was conducted on the handling practices of *Kayabo* from the two locations. Three clusters were obtained; cluster I (15 companies), cluster II (10 companies) and cluster III (5 companies). All cluster I processors were from Kanyama, whereas the rest of clusters contained processors from Mwaloni. The study showed that, *Kayabo* value chain was associated with poor quality raw material, inadequate handling practices, lack of food grade processing equipment, poor sanitation, lack of training and education on hygiene as well as poor packaging and storage conditions. All clusters exhibited TVC, *S. aureus*, total coliforms with values ranging from 2.08 - 8.68 Log CFU/g, 2.36 – 2.56 Log CFU/g, 0.6 – 1.36 Log MPN/g, respectively. Only TVC indicated significant difference ($P<0.05$) among the clusters. However, *E. coli* and *L. monocytogenes* were not detected in all samples tested.

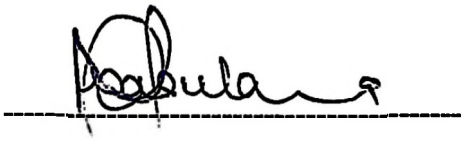
Kayabo from the two locations varied significantly ($P<0.05$) in moisture content, TVB-N and TMA-N contents. Samples from Mwaloni had highest mean scores of moisture content (42.8%) compared to Kanyama (27.4%). The moisture content of *Kayabo* from Mwaloni exceeded the 30% cut-off point for the dried salted fishery products. All samples from Mwaloni had TMA-N and TVB-N values within the acceptable limit. The higher concentration of TVB-N and TMA-N recorded beyond the freshness scale from majority of *Kayabo* samples from Kanyama location gave an indication that the products were made from the raw materials which had started to spoil.

The study showed that preservation of *Kayabo* with salt has varied effect on both quality and safety parameters. The fact that salt can inhibit growth and proliferation of spoilage and pathogenic microorganisms i.e. *L. monocytogenes*, *S. aureus* and *E. coli*, it is therefore recommended that it is appropriately used. However, since growth of some spoilage microorganisms was observed; it is further recommended to improve the

handling practices by using cold storage from the fish supply sources to the processing units, hygiene training and education, inspection of *Kayabo* processing facilities and operations, adequate drying of *Kayabo*, appropriate final product packaging, labeling and storage in order to improve significantly safety and quality of *Kayabo*.

DECLARATION

I, Nuru Edgar Mwasulama do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is the result of my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.



6/11/2017

Nuru Edgar Mwasulama — Date

(MSc. Food Quality and Safety Assurance Candidate)

The above declaration is confirmed by;

6/11/2017

Prof. B. P. M. Tiisekwa — Date

(Supervisor)

6/11/2017

Dr. J. B. Kussaga — Date

(Supervisor)

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DEDICATION

To my parents, husband Jeremiah Joe Kabissa, daughter Joenice-Tusajigwe J. Kabissa, and son Joshua-Gwamaka J. Kabissa whose daily prayers and encouragement enabled me to complete this study. To my beloved late uncle Mr. Nelson Amulike Mwaijengo; who believed in me, paid for my education and took care of me.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
<	Less than
>	Greater than
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ATP	Adenosine triphosphate
a_w	Water activity
BPW	Buffered Peptone Water
CAC	Codex Almentarius Commission
CDR	Corporate Document Repository
CFU	Colony Forming Units
DFTNCS	Department of Food Technology, Nutrition and Consumer Sciences
DHA	Decosahexanoic Acid
DMA	Dimethylamine
EAC	East African Community
EACM	East African Common Market
EAS	East African Standards
EC	European Commission
EPA	Eicosapentanoic Acids
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA - BAM	Food and Drugs Administration – Bacteriological Analytical Manual

g	gramme
GCLA	Government Chemist Laboratory Agency
GDP	Gross Domestic Product
GHP	Good Hygienic Practices
GMP	Good Manufacturing Practices
HCl	Hydrochloric acid
ISO	International Organization for Standardization
Kg	Kilogramme
LCPUFA	Long Chain Polyunsaturated Fatty Acids
LVFO	Lake Victoria Fisheries Organization
M	Molarity
MAL	Maximum Allowable Limits
MC	Moisture Content
N	Normality
NaCl	Sodium chloride
NFQCL	National Fish Quality Control Laboratory
PUFA	Poly-unsaturated Fatty Acids
RH	Relative Humidity
SPSS	Statistical Package for Social Sciences
SSO	Specific Spoilage Organisms
TBS	Tanzania Bureau of Standards
TFDA	Tanzania Food and Drugs Authority
TFDCA	Tanzania Food, Drugs and Cosmetics Act
TMA-N	Trimethylamine Nitrogen
TMAO	Trimethylamine Oxide
TVB-N	Total Volatile Basic Nitrogen

TVC	Total Viable Counts
TZS	Tanzanian Standards
URT	United Republic of Tanzania
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Fish is a good source of high quality proteins, vitamins and minerals. It is relatively cheaper than red meat; thus, it is used as a substitute to meat (Thilsted *et al.*, 2013). Fatty fish species have high content of Long Chain Polyunsaturated Fatty Acids (LCPUFAs), which are associated with improving health and preventing diseases in human beings. However, fish is among the perishable products because of high water content, protein and inherent microorganisms. The post-harvest losses of fish are enormously high (5 – 25 % in Sub-Saharan Africa) which contribute to both food and nutritional insecurity (Kabahenda *et al.*, 2011; FAO, 2014). A review of case studies on post-harvest losses in Sub-Saharan Africa including Tanzania indicates high levels of losses both in quantity and quality of fishery products (Matís, 2010; Kabahenda *et al.*, 2011; FAO, 2014; Getu *et al.*, 2015). Due to high content of LCPUFAs, fish products are very susceptible to oxidation. Oxidation of lipids is associated with a decrease in triacylglycerols and phospholipids and an increase in free fatty acids and often results in a product with off flavors (rancid) which may not be appealing to consumers. Fatty fish species such as Nile perch (*Lates niloticus*) have high levels of Polyunsaturated Fatty Acids (PUFAs), especially the 2-3 fatty acids Eicosapentanoic Acid (EPA) and Docosahexanoic Acid (DHA), which are very susceptible to rancidity. Moreover, fish is rich in protein (15 – 20 %) which makes it susceptible to rapid degradation by micro-organisms if not properly handled (Kabahenda *et al.*, 2011; FAO, 2014). Fish is thus, a product that needs proper handling and processing in order to preserve nutrients and its functional components that promote good health. Lowering the temperature of fresh fish by icing as soon as it is harvested has been observed to be a cost-effective method of fish

preservation (Okeyo and Lokuruka, 2010). Therefore, ensuring cold chain in fish processing will prevent spoilage and extend the shelf life. The shelf life of fresh fish could be extended to more than 4 days in ice (Okeyo *et al.*, 2009; Okeyo and Lokuruka, 2010).

Volatile amines are the characteristic molecules responsible for the fishy odour and flavour present in fish and they are commonly used as criteria for assessing fish quality (Wu and Bechtel, 2008). Total Volatile Bases Nitrogen (TVB-N) is one of the most widely used methods to estimate the degree of decomposition of fish. It includes the measurement of trimethylamine (TMA-N) (produced by spoilage bacteria), dimethylamine (DM) (produced by autolytic enzymes during frozen storage), ammonia (NH_3) (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile nitrogenous compounds associated with seafood spoilage (Castro *et al.*, 2006; Wu and Bechtel, 2008). Trimethylamine (TMA-N) provides an accurate indication of bacterial spoilage of fish (El- Marrakch *et al.*, 1990; Fathia and Abdul, 2013). It is a pungent volatile amine often associated with the typical fish odour of spoiling fish and fishery products. Its presence in spoiling fish is due to the bacterial reduction of trimethylamine oxide (TMAO) which is naturally present in the living tissue of many marine fish species (Immaculate *et al.*, 2013).

Fish processing companies in Tanzania are basically for the export market with strict control and monitoring of the food safety hazards including chemical (i.e. antibiotics, pesticides, dynamites) and biological (microbiological and parasites) (Kussaga, 2015). Industrial fish processing for the domestic market is very limited (Kussaga, 2015). The major processors of fish for the domestic market are small scale processors often with limited knowledge on proper handling and inadequate hygienic facilities to ensure

quality and safety of the products (Kussaga, 2015). Lack of enough raw materials for the small scale processors of fish for the domestic market compels them (including *Kayabo* processors) to purchase rejects (undersize, poor quality and fish frames) from fish processing companies. Apart from rejects from fish processing companies that process fish for the export market, *Kayabo* processors obtain their raw materials (often spoiled) from artisanal fisheries and landing sites fish rejects. The current trend in utilization of fish by-products is not only a move to reduce wastes but also a strategy to improve availability of fish to consumers who cannot access high-value fish such as Nile perch fish fillets (Kabahenda, 2009). Use of rejects and poor quality raw materials associated with inadequate handling and processing conditions could result into food safety problems.

1.2 Problem Statement and Justification

Although fish contributes to food and nutritional security, they are very perishable and prone to microbiological contamination (Kussaga, 2015). Inadequate processing conditions could contribute to cross contamination and growth of inherent microorganisms including pathogens that could limit shelf-life and cause foodborne diseases (Mbunda, 2012; Roesel *et al.*, 2015). If not properly handled (lack of cold chain), fish could easily spoil soon after capture. During storage characteristic flora develops, but only a part of this flora, known as the specific spoilage organisms (SSO), contribute to spoilage (Amos, 2007). Examples of SSO are *Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Pseudomonas* spp. and *Vibrionaceae* spp.

Rejects from landing sites and fish processing companies for the export market constitute the raw materials for processing of low-value products including *Kayabo*. These rejects are not properly handled and could spoil before reaching the *Kayabo* processing units (Kabahenda, 2009). Besides lack of cold chain along the *Kayabo* processing chain,

illegally fished Nile perch (the use of illegal fishing gears e.g. poisonous chemicals and dynamites) could find their way to these processing units. In addition, failure to apply adequate quality and safety measures may lead to significant post-harvest losses of fish along the processing chain. In general, *Kayabo* is exposed to various food safety hazards that could result into food borne diseases.

Although various studies on quality and safety of high value fish products for the export market have been performed (Kussaga, 2015), there is limited studies on quality and safety of low-value fish products including *Kayabo* for the domestic market. The purpose of this study is to assess microbiological and chemical quality of *Kayabo* and current hygienic practices of processors.

The findings from this study will serve as a basis for improving the current situation along the *Kayabo* processing value chain. In addition, the findings will provide the microbiological and chemical status of *Kayabo* which will be useful to food control authorities to develop strategies to assure safety of the products. Improving processing and storage facilities of *Kayabo*, will in turn assure safety and quality of the product as well as promoting public health, income generation, and market expansion.

1.3 Overall Objective

The overall objective of the study was to assess the microbiological and chemical quality of *Kayabo* – a salted sundried *Lates niloticus* from Lake Victoria.

1.3.1 Specific objectives

i. To assess the current handling practices of *Kayabo* along the processing chain.

ii. To assess the microbiological quality of *Kayabo*.

iii. To determine the chemical quality of *Kayabo*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Role of Fisheries to Tanzania Economy

Fishery sector provides substantial employment, income, livelihood, foreign earnings and revenue to the country (United Republic of Tanzania, 2013). The fisheries sector employs more than 400 000 full time artisanal fishermen in the country (Abila, 2003; Abila and Konstantine, 2006). More than 4 000 000 people make their livelihoods through various fisheries-related activities (United Republic of Tanzania, 2013). The fish industry contributes about 1.3% to GDP and about 30% of the animal protein consumed in the country, as well as the sole source of raw material for the fishery processing companies including *Kayabo* processors (United Republic of Tanzania, 2013). Fish industry is the leading food exporting sector and among the major sources of foreign exchange in the country (United Republic of Tanzania, 2013).

2.2 Post – harvest Fish Handling

It has been estimated that 10 percent by weight of world fish catch is lost by poor handling, processing, storage and distribution (Akande and Diei-Ouadi, 2010). However, losses in small scale fish processing are estimated to be 5 – 25 % in Sub-Saharan Africa (Akande and Diei-Ouadi, 2010). Enzymatic and bacterial spoilage of fish can be reduced or temporarily halted by various techniques, including temperature reduction by the use of ice, drying to reduce or completely remove water, salting to reduce water and stop enzymatic decomposition (Tawari and Abowei, 2011). In Tanzania, fish is mainly consumed fresh or processed (smoked, sun-dried, and salted-sun dried) (United Republic of Tanzania, 2013). FAO economic data for the United Republic of Tanzania indicates an average of US$13 million post-harvest losses of fish and fish products (FAO, 2014) .

2.3 Fish Spoilage Mechanisms and Preservation Techniques

Spoilage of food products is due to chemical, enzymatic or microbial activities (Ghaly *et al.*, 2010). The freshness and quality of fish have always gained the attention by food regulatory agencies and fish processing companies, as well as consumers (Patterson *et al.*, 2012). Proper handling, pretreatment and preservation techniques can improve the quality fish and fish products and increase their shelf life (Ghaly *et al.*, 2010).

Fish spoilage could be induced through enzymatic autolysis, oxidation, and / or microbial growth. Fresh fish spoilage occurs very rapid in the tropics. The spoilage occurs within 12 hours of capture (Berkel *et al.*, 2004 and Ghaly *et al.*, 2010). Fish spoilage involves various mechanisms i.e. enzymatic autolysis, oxidation, and microbial growth (AMEC, 2003). Spoilage of fish is associated with breakdown of various components and the formation of new compounds responsible for odour, flavor and textural changes of the fish meat (Immaculate *et al.*, 2013).

2.3.1 Fish spoilage mechanisms

2.3.1.1 Autolytic enzymatic spoilage

Shortly after capture chemical and biological changes take place in dead fish due to enzymatic breakdown of major fish molecules (FAO, 2005). A number of proteolytic enzymes are found in muscle and viscera of the fish after catch. These enzymes contribute to post mortem degradation in fish muscle and fish products during storage and processing (Ghaly *et al.*, 2010).

Table 2.1: Summary of changes in chilled or frozen fish

Enzyme(s)	Substrate	Effect	Prevention
Glycolytic enzymes	Glycogen	Lactic acid production resulting in pH drop	Avoid pre-rigor stress
Autolytic enzymes involved in nucleotide breakdown	ATO, ADP, AMP, IMP	Gradual production of Hypoxanthine	Avoid pre-rigor stress
Cathepsins	Proteins, peptides	Softening of tissues	Avoid rough handling during storage
Chymotrypsin, trypsin, carboxy-peptidases	Proteins, peptides	Belly-bursting	Problem increased with freezing/thawing or long-term chill storage
Calpain	Myofibrillar proteins	Softening	Removal of calcium
Collagenases	Connective tissue	Softening and gaping of tissue	Time and temperature of chilled storage
Trimethylamine Oxide (TMAO) demethylase	TMAO	Formaldehyde	Storage temperature less than -30^{0}C, physical abuse, freez/thawing

Adapted from FAO (2005)

2.3.1.2 Oxidative spoilage

Lipid oxidation is a major cause of deterioration and spoilage of fish species with high oil/fat content e.g. *Lates niloticus* (Ghaly *et al.*, 2010). Lipid oxidation involves a three stage free radical mechanisms: initiation, propagation and termination. Initiation involves the formation of lipid free radicals through catalysts such as heat, metal ions and irradiation. These free radicals react with oxygen to form peroxyl radicals. During propagation, the peroxyl radicals react with other lipid molecules to form hydroperoxides and a new free radical (Amos, 2007). Termination occurs when a build-up of these free

radicals interact to form non-radical products. Oxidation typically involves the reaction of oxygen with the double bonds of fatty acids.

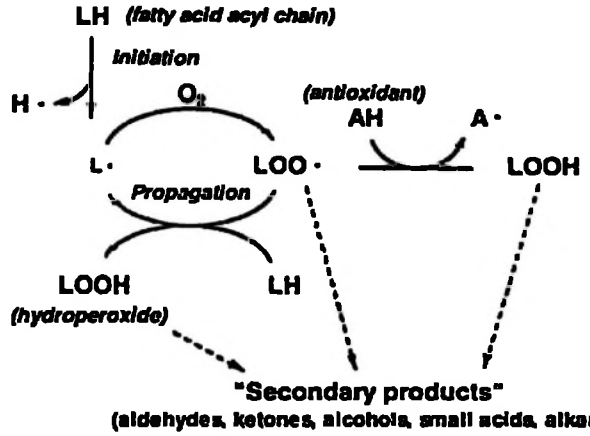

Figure 2.1: Autoxidation of polyunsaturated lipids (FAO, 2005)

2.3.1.3 Microbiological spoilage

The flesh of healthy live or newly caught fish is normally considered to be sterile, but microorganisms are found on all the outer surfaces (skin and gills) and in the gut (Ghaly *et al.*, 2010). Composition of the micro flora on a newly caught fish depends on the microbial contents of the water in which the fish live (Amos, 2007). Fish from contaminated water can contain 10^2-10^7 CFU/cm^2 on the skin and between 10^3 and 10^9 CFU/g in the gills and intestines (Amos, 2007).

When fish dies, its entire body resistance mechanisms breakdown, giving way to microorganisms or the enzymes they secrete to invade or diffuse into the flesh where they react with the complex mixture of natural substances present (Ghaly *et al.*, 2010). During storage a characteristic flora develops, but only a part of this flora, known as the specific spoilage organisms (SSO), contribute to spoilage (Amos, 2007). The SSO counts reach a minimal spoilage level where the fish is sensorially rejected (Figure 2.2). Temperate fish have psychrotrophic (cold-tolerant) bacteria of the genera *Pseudomonas*,

Moraxella, Acinobacter, Shewanella Flavobacterium, Vibrio, Photobacterium and *Aeromonas* as part of their natural flora whereas tropical fish normally have non-psychrotrophic (mesophilic) spoilage bacteria that make tropical fish spoil much faster than temperate water fish in the absence of ice (Amos, 2007).

These bacteria can obtain their energy by reducing and decomposing TMAO, proteins and other nitrogen containing compounds. Microbial growth and metabolism is a major cause of fish spoilage which produce amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable off-flavors (Ghaly *et al.*, 2010). Trimethylamine oxide (TMAO) is broken down to trimethylamine (TMA), dimethylamine (DMA) and ammonia (NH_3). Trimethylamine (TMA) levels are used universally to determine microbial deterioration leading to fish spoilage (Patterson *et al.*, 2012). Fish use Trimethylamine Oxide (TMAO) as an osmoregulant to avoid dehydration in marine environments and tissue waterlogging in fresh water.

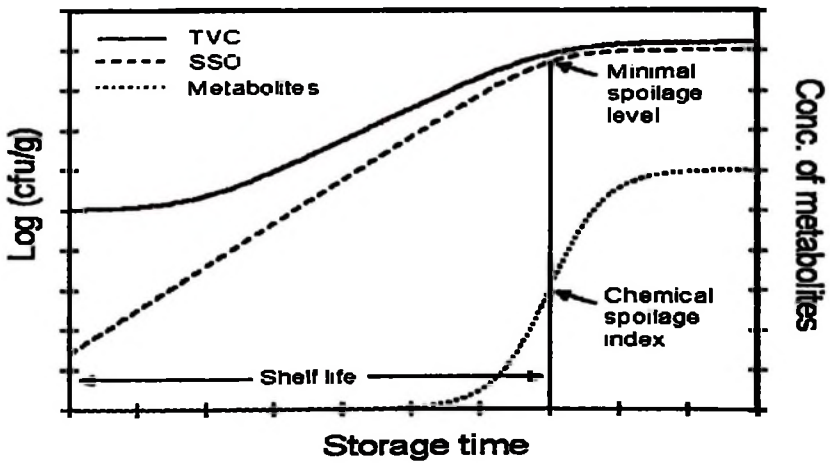

Figure 2.2: General pattern of microbial spoilage changes in total viable count (TVC), specific spoilage organisms (SSO), and chemical spoilage indices during chilled storage of a fish product (Adapted from Ryder *et al.*, 2014).

2.3.2 Fish preservation techniques

Different types of preservation methods such as drying, smoking, freezing, chilling, brining, fermentation and canning are reported to extend the shelf-life of fish and fishery products (Quang, 2005; Ghaly *et al.*, 2010 and Ayinsa and Maalekuu, 2013).

2.3.2.1 Low temperature storage

Since mid of 19th century, the low temperature storage methods have been used for the preservation of wide varieties of sea foods which retard the growth of microorganisms (Ghaly *et al.*, 2010). This method of preservation does not kill the microorganisms but reduces microbial metabolism which is responsible for spoilage. Berkel *et al.* (2004) reported two possibilities for storing fresh fish at low temperatures; (a) cooling at -1°C to +4°C, which inhibits the growth of microorganisms and (b) freezing at -18 to -30°C, which completely stops bacteria from growing. However, both enzymatic and non-enzymatic changes continue but at a much slower rate. About 10-60% of the viable microbial population die during freezing yet the remaining population gradually increase during frozen storage (Berkel *et al.*, 2004).

2.3.2.2 Controlling water activity (a_w)

Fish spoilage can also be prevented by controlling water activity (Abbas *et al.*, 2009). For the growth of every microorganism there are minimum, optimum and maximum water activity same like pH and temperature. Lowering water activity (a_w) can minimize putrefaction and improve preservation of fish (Abbas *et al.*, 2009; Ghaly *et al.*, 2010). The term water activity (a_w) refers to the water which is not bound to food molecules and can support the growth of bacteria, yeasts and moulds (Ghaly *et al.*, 2010). The water activity (a_w) represents the ratio of the water vapor pressure of the food to the water vapor pressure of pure water under the same conditions and it is expressed as a fraction.

The control of water activity (a_w) in fish is accomplished by drying, adding chemicals, or a combination of both methods (hurdles). Sugars and sodium chloride have been used to bind up the free water molecules and create an osmotic imbalance resulting in cell growth inhibition (Ghaly *et al.*, 2010, Dave and Ghaly, 2011).

It was reported that the inhibitory effects of sucrose and sodium chloride on *Staphylococcus aureus* were primarily related to lower water activity (Dave and Ghaly, 2011). Comparing the minimal water activity for growth of various pathogenic bacteria using sodium chloride or glycerol, in all cases sodium chloride was found to be more inhibitory than glycerol. Salting decreases water activity and has inhibition effect on pathogenic bacteria (Teklemariam *et al.*, 2015).

Wijnker *et al.* (2006) studied antimicrobial properties of salt (NaCl) for the preservation of natural sheep casings at different water activity (a_w) levels and found the activities of most spoilage and pathogenic bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus* and *E. coli* O157:H7) stopped when an a_w of 0.89 was reached. Micro-organisms generally grow best between a_w values of 0.980-0.995 and growth ceases at a_w<0.9 (Teklemariam *et al.*, 2015).

2.3.2.3 Controlling autolytic enzymatic spoilage

As the fish degradation process begins with autolytic activity, it is important to slow the action of the digestive enzymes to improve preservation. This can be accomplished by removing the enzymes or by developing techniques that inhibit their activities. Gutting of the fish immediately after capture can avoid the invasion of digestive tract proteases through the abdominal cavity to the tissue and prevent or slow degradation (Ghaly *et al.*, 2010). Sodium chloride has shown the ability to inactivate autolytic enzymes (e.g.

cathepsins) and catheptic activity in marine species (Ghaly *et al.*, 2010). About 25% (w/w) NaCl reduce autolytic activity by 48% (Fig. 2.3).

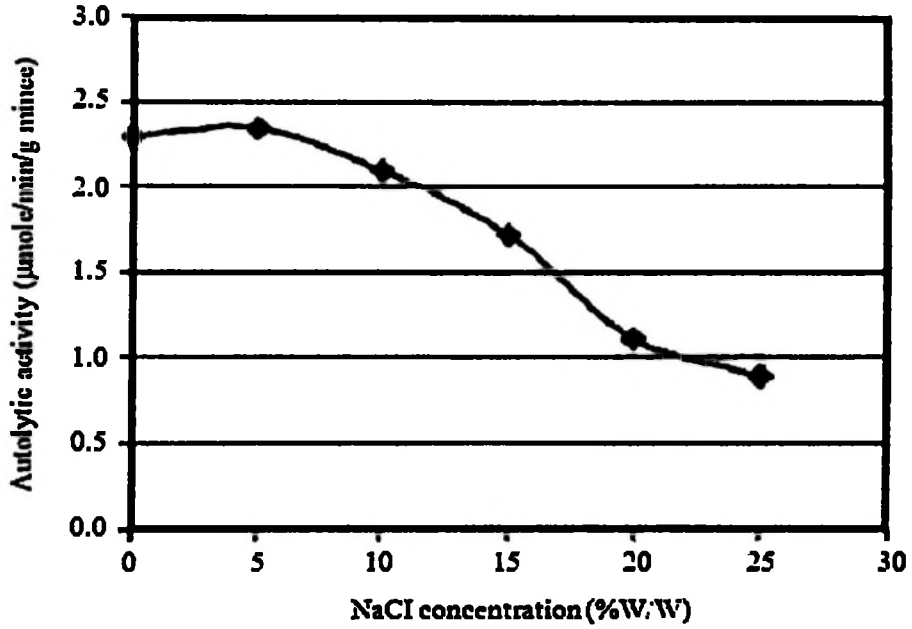

Figure 2.3: Effect of NaCl on autolytic activity in Indian anchovy (Ghaly *et al.*, 2010)

2.4 *Kayabo* Handling and Processing Methods

The major factors that affect the nutritive value of fish products are related to how fish is handled, processed and stored (Getu *et al.*, 2015). Traditional processing (solar drying, curing) and handling practices (lack of cooling, covering and poor hygiene) subject fish to various spoilage agents (Kabahenda, 2009). The by-products from filleting factories are even at greater risk of spoilage because they are often treated as wastes. By-products undergo tissue damage during processing, have large surface area of flesh exposed to atmospheric air, and are stored at ambient temperatures, all of which are key factors that determine the rate and extent of rancidity (Kabahenda, 2009).

2.5 Quality of Cured Fish Products

Small-scale fish processors sun-dry fish either without salt or rub in dry salt to process low-grade fish products i.e. *Kayabo* (Kabahenda, 2009). They use the traditional technologies when the fish catch cannot be marketed immediately after capture either due to glut, size or type of fish species caught. A common problem with sun-drying unsalted fish in the tropics is infestation with the dermestid beetles, although natural pyrethrin extracts have been used successfully to solve the problem in Kenya (Lokuruka, 2002).

Salted–sundried fish products are highly appreciated because of storage stability, nutritional stability and their characteristic taste, texture and aroma (Nguvava, 2013). Nevertheless, cured products have the advantage of being storable for periods of up to one year or longer if they are free of insect infestation, properly packaged, and stored in sanitary conditions of less than 60% RH (Mbunda, 2012).

2.5 Quality and Safety Assessment of Fish and Fishery Products

Several methods are used to determine the quality and safety of fish products. These can be classified into sensory and instrumental methods. The latter comprises of chemical, physical and microbiological methods. The most widely used chemical test is total volatile bases (TVB) or total volatile basic nitrogen (TVBN), which measures the content of trimethylamine (TMA) + dimethylamine (DMA) + ammonia + other basic nitrogenous compounds associated with fish spoilage. Other tests target the separate measurement of TMA, DMA, nucleotide catabolites (known as the %K-value) or biogenic amines contents (BA) (Dergal *et al.*, 2013). The K or "freshness" index gives an indication of fish freshness during the early stages after capture, whereas TMA, TVB or BA gives this indication at later stages when bacterial spoilage starts. Dimethylamine

(DMA) is used to measure the quality of frozen fish. Also, oxidative rancidity is measured by evaluating the peroxide value (PV) during the early stages or the thiobarbituric acid-related substances (TBA-RS) during later stages (Dergal *et al.*, 2013).

Physical methods involve the measurement of fish muscle pH, texture or electrical properties. These methods are rarely used because they are either not sufficiently reliable or require calibration depending on the fish species (Codex, 1999).

Microbiological examination of fish aims at evaluating hygienic quality of fish, including temperature abuse, and the possible presence of pathogenic microorganisms in the fish. They mainly consist of the measurement of total aerobic bacteria also called total plate count, spoilage bacteria, and various pathogenic bacteria. Although all these methods mainly assess quality characteristics of fish and fishery products, quality and safety are correlated (Dergal *et al.*, 2013).

2.5.1 Microbiological and chemical criteria of fish and fishery products

2.5.1.1 Microbiological criteria

A microbiological criterion is a standard against which comparison and assessment of research data may be made. The standard may have either obligatory or optional status. A microbiological *standard* is a microbiological criterion that is part of a law or ordinance and is an obligatory criterion. A microbiological specification is used in purchase agreements between buyer and vendor (FAO/CDR, 2013). Microbiological criteria may be useful in evaluating the safety and shelf-life of foods, the adherence to established Good Manufacturing Practices (GMP) and the correctness of food for a specific purpose (FAO/CDR, 2013;Teklemariam *et al.*, 2015). The microbiological parameters carried out in this study were the enumeration of total viable counts / total plate counts (TPC), *Escherichia coli*, total coliforms, and *Staphylococcus aureus* as well

as detection of *Listeria monocytogenes*. The test methods used and microbiological criterion are indicated in Table 2.2.

Tests for TVC is useful for measuring the conditions of the raw material, effectiveness of the handling procedures and hygiene conditions during processing, sanitary conditions of equipment and utensils and time/temperature profile during storage and distribution. The natural habitat for *E. coli* is the intestines of human and vertebrate animals. Contamination of food with *E. coli* implies a risk that one or more of enteric pathogens may have gained access to the food (FAO / CDR, 2013). Total coliforms indicate a higher probability to contain organisms of fecal origin and hence indicating fecal contamination. The natural reservoir for *S. aureus* is human skin, hair and superficial mucous membranes (nose), presence of large numbers indicate the possible presence of enterotoxin and/or fault in sanitary or production practices. The natural habitation of *L. monocytogenes* is in intestines of vertebrate animals, soil, untreated water, and fecal matter. Its presence in food is an indication of poor sanitation and animal control (FAO / CDR, 2013).

Table 2.2: Test methods and microbiological criteria of fish and fishery products

Parameter	Test method	Criteria (maximum limit)	Source
Total Viable Count (TVC)	ISO 4833:2003 (TZS 118:2007) Microbiology of food and animal feeding stuffs- Horizontal Method for enumeration of microorganisms- Colony-count technique at 30^{0}C	1 x 10^5 CFU/g	TZS 118:2007 EAS 828:2016
E.coli	ISO 7251:2005 (TZS 731:2007) Microbiology of food and animal feeding stuffs- Horizontal Method for the detection and enumeration of presumptive *E. coli* - Most probable number technique	1 x 10^1 MPN/g Absent	TZS 731:2007 EAS 828:2016 CAC/RCP 52-2003
Total coliforms	ISO 4831:2006 (TZS 119:2002) Microbiology of food and animal feeding stuffs- Horizontal Method for detection and enumeration of coliforms - Most probable number technique	4 x 10^2 MPN/g	TZS 119:2002
S. aureus	ISO 6888 – 3: 2003 (TZS 125:2002) Microbiology of food and animal feeding stuffs- Horizontal Method for Enumeration and Detection of coagulase-positive staphylococci(*Staphylococcus aureus*) and other species - Part 1: Technique using BPA	1 x 10^3 CFU/g 2 x 10^3 CFU/g	TZS 125:2002 EAS 828:2016
L. Monocytogenes	ISO 11290-1:1996/Amd 1: 2004 Microbiology of food and animal feeding stuffs- Horizontal method for the detection and enumeration *L. monocytogenes*- Part 1: Detection method	Absent in 25g	CAC/RCP 52

2.5.1.2 Chemical criteria (TVB-N and TMA)

The combined total amount of ammonia (NH_3), dimethylamine (DMA) and trimethylamine (TMA) in fish is called the total volatile base (TVB) nitrogen content of the fish and is commonly used as an estimate of spoilage and has been widely used as an index for freshness of fish (Wu and Bechtel, 2008; Jinadasa, 2014). Total volatile basic nitrogen (TVB-N) is important characteristic for the assessment of quality in seafood products and appears as the most common chemical indicators of marine fish spoilage (Dalgaard *et al.*, 1993; Amegovu *et al.*, 2012; Wu and Bechtel, 2008). A European Union directive on fish hygiene specifies that if the organoleptic examination reveals any

doubt as to the freshness of the fish, inspectors must use TVB-N as a chemical check (Castro *et al.*, 2006).

The level of TVB-N for white fish is generally considered to be fresh if the TVB-N is less than 20 mg/100g sample, beginning of spoilage (stale) when 20 – 30mg/100g and spoiled fish when >30mg/100g (Jinadasa, 2014 and El-Sherif, 2016) . This suggests that fish and fish products are unfit for human consumption when TVB-N exceeds 30 mg N/100g flesh.

Trimethylamine nitrogen (TMA-N) is often used as an index in evaluating the shelf-life and keeping quality of fish products because it rapidly accumulates in the muscle under refrigeration conditions (Fathia *et al.*, 2013). The TMA-N production in fish tissue during cold storage could be used as an indicator of bacterial activity and is an accepted measure of deterioration. The pungent odour of spoiled fish has been related to the TMA-N tissue levels also with the number of spoilage organisms present in many fish species (Fathia *et al.*, 2013). The rejection limit is usually from 5 – 10 mg TMA-N / 100 g muscle (El- Marrakch *et al.*, 1990 ; Fathia *et al.*, 2013).

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CHAPTER THREE

PAPER ONE

3.0 HANDLING PRACTICES AND MICROBIOLOGICAL QUALITY OF *KAYABO* - A SALTED SUN-DRIED NILE PERCH *Lates niloticus* FROM LAKE VICTORIA, TANZANIA

Nuru E. Mwasulama[1], Jamal B. Kussaga[2], Bendantunguka P. M. Tiisekwa[2]

[1]Tanzania Food and Drugs Authority, P. O. Box 77150, Dar es Salaam, Tanzania
E-mail: nurumwasulama@gmail.com

[2]Department of Food Technology, Nutrition and Consumer Sciences, Sokoine University of Agriculture, P. O. Box 3006, Morogoro, Tanzania
E-mail of the corresponding author: kussaga@suanet.ac.tz

[2]Department of Food Technology, Nutrition and Consumer Sciences, Sokoine University of Agriculture, P. O. Box 3006, Morogoro, Tanzania
E-mail: tiisekwa@suanet.ac.tz

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3.1 Abstract

The study aimed at assessing the microbiological quality and handling practices of a salted sun-dried *Lates niloticus* (*Kayabo*) from Lake Victoria, Tanzania. Structured observation checklist was used to collect information from 30 *Kayabo* processors on the handling practices of the salted sun-dried *Lates niloticus* along the processing chain. Samples were collected from processors from two Municipalities of Mwanza City namely Nyamagana (Kanyama) and Ilemela (Mwaloni). Hierarchical cluster analysis on the handling practices of *Kayabo* from the two locations produced three clusters composed of 15 (cluster I), 10 (cluster II) and 5 (cluster III) companies. All cluster I processors were from Kanyama, whereas the rest of clusters contained processors from

Mwaloni. The study showed that, *Kayabo* value chain was associated with poor quality raw material, inadequate handling practices, lack of food grade processing equipment, poor sanitation, lack of training and low education on hygiene as well as poor packaging and storage conditions. Assessment of microbiological quality of *Kayabo* indicated that TVC was quantified in all clusters with values ranging from 2.08 - 8.68 Log CFU/g. Cluster I had 12 out of 15 processors exceeding the maximum allowable limit (5 Log CFU/g) of TVC. *Staphylococcus aureus* were quantified in 12/15 companies (with 5 companies exceeding the allowable limit of 3 Log CFU/g) in cluster I, 1/10 companies in cluster II and 1/5 companies in cluster III. However, *Escherichia coli* and *Listeria monocytogenes* were not detected in all samples tested. Although TVC, total coliforms and *S. aureus* were excessively quantified in all clusters, only TVC indicated significant difference ($P<0.05$) among the clusters. Despite the fact that most of the processors operate in very inadequate situations, there were no pathogens detected, this could be due to the use of salt which inhibit growth of most pathogens. To ensure microbiological quality of *Kayabo*, processors are advised to ensure use of quality raw materials, good manufacturing, hygienic and distribution practices.

KEY WORDS: fish, salted sundried Nile perch, fish handling practices, microbiological quality, *Kayabo*, Lake Victoria.

3.2 Introduction

Fish is among the protein rich foods; among the excellent dietary sources of highly unsaturated fatty acid (UFA) and polyunsaturated fatty acid (PUFA) especially the omega-3 fatty acids, Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) (El-Sherif and Abd El-Ghafour, 2016), minerals (i.e. iron, calcium, iodine and potassium) and vitamins including vitamin A, vitamin B_2, vitamin B_6 (Akande and Diei-

Ouadi, 2010; Kawarazuka, 2010; Tacon and Metian, 2013). It is therefore contributing to food and nutrition security.

Tanzanian fishery sector provides substantial employment, income, livelihood, foreign earnings and revenue to the country (FAO, 2014; United Republic of Tanzania, 2013). It is estimated that about 30% of the total animal protein requirements come from fish (United Republic of Tanzania, 2013). The fish industry contributes about 1.3% GDP (Environment, 2002; FAO, 2014; LVFO, 2009 and URT, 2013) and employs more than 400 000 full time artisanal fishermen in the country and more than 4 000 000 people make their livelihoods through various fisheries-related activities (United Republic of Tanzania, 2013). Fish industry is the leading food exporting industry and among the major sources of foreign exchange in the country. Lake Victoria is a very important source of fresh water fish in East Africa. It has more than 290 fish species and among these, the commercially important ones in Tanzania include Nile perch (*Lates niloticus*), Tilapia (*Oreochromis niloticus*) and Sardines (*Rastrineobola argentea*), the small-sized fish species (Abila, 2000; Akande and Diei-Ouadi, 2010; Kabahenda, 2009 and Mhongole, 2009).

Industrial fish processing in Tanzania is basically for the export market with strict control and monitoring of the food safety hazards (Kussaga, 2015). Small-scale processors often with limited knowledge on proper handling and inadequate hygienic facilities to ensure quality and safety of the products dominate the domestic market (Kussaga, 2015). Curing and sun-drying are among the major processes undertaken by the small-scale processors. Lack of enough raw materials for the small-scale processors of fish for the domestic market (including *Kayabo* processors) compels them to purchase rejects (undersize, poor quality and fish frames) from fish processing companies

(Kabahenda, 2009). Apart from rejects from fish processing companies for the export market, *Kayabo* processors also obtain their raw materials (often spoiled due to absence of cooling) from artisanal fisheries and landing sites fish rejects. The use of rejects and poor quality raw materials associated with inadequate handling and processing conditions could result into food safety problems.

Fish is made up of 70-84 percent water, 15-24 percent protein, 0.1-22 percent fat and 1-2 percent minerals (Ghaly *et al.*, 2010). Unless preserved or processed, fish is highly perishable product; that can be rendered unfit for human consumption within twelve hours of capture under tropical conditions (Ghaly *et al.*, 2010; Nguvava, 2013). Immediately after fish dies, a number of physiological and microbial deterioration set thereby degrade the fish (Davies, 2009; Sulieman *et al.*, 2012; Jinadasa, 2014). Hygienic handling practices should be employed to extend its shelf life (FAO, 2004; Ghaly *et al.*, 2010 and Nguvava, 2013). However, the quality of fish and its usefulness is affected by the capture method, handling practices, processing methods, distribution techniques and storage (Akande and Diei-Ouadi, 2010; Amos, 2007; Matís, 2010; Mbunda, 2012; Nguvava, 2013 and Quang, 2005). Lack of adequate knowledge on proper handling and post-harvest practices contributed to the poor quality of fish and fishery products including *Kayabo* (FAO, 2013).

Although various studies on quality and safety of high value fish products for the export market have been conducted (Kussaga, 2015), there is limited information on the quality and safety of low-value fish products including *Kayabo* for the domestic and regional markets. It is not known to what extent consumers of fish from the local market are exposed to various food safety risks. Therefore, the purpose of this study was to investigate microbiological quality and handling practices of *Kayabo* along the value

chain. The information generated from this study will be beneficial to consumers, small scale processors, researchers, food control authorities and policy makers to set strategies to improve food processing practices of the small-scale processors and reduce the risk of consumers exposed to various food safety risks.

3.3 Materials and Methods

3.3.1 Study area

The study was conducted in two Municipalities of Mwanza City, namely; Nyamagana (Kanyama area) and Ilemela (Mwaloni market). The areas were purposively selected because they contain majority of *Kayabo* processors in the region. The systematic random sampling was used to pick *Kayabo* processors. Since the exact population of *Kayabo* processors were not known, the sample size was estimated by using Kothari equation (Kothari and Garg, 2014).

3.3.2 Characteristics of the small-scale fish processors involved in the study

Thirty *Kayabo* processors, 18 being micro-scale (<10 employees) and 12 small-scale (10–49 employees) participated in this study. These companies produced whole salted sun-dried Nile perch (*Kayabo*), salted sun-dried Nile perch heads (*Mapanki*), salted sun-dried Nile perch trims (chips) and sun-dried Nile perch bones for fish meal production.

3.3.3 Assessment of hygienic practices

The hygienic practices (transportation, processing conditions, packaging and storage of raw materials and products) of the companies involved in the study were analyzed by using data collected from closed ended questionnaire (Appendix 1). The questionnaires were administered to the randomly selected 30 processors (15 from Kanyama and 15 from Mwaloni).

3.3.4 Microbiological assessment

3.3.4.1 Selection of microbiological parameters

Five microbiological parameters including indicators of general process hygiene (Total Viable Counts, TVC), faecal hygiene (total coliforms and *Escherichia coli*), personnel hygiene (*Staphylococcus aureus*) and food safety (*Listeria monocytogenes*) were selected and analyzed. These indicators were selected because they are not part of the normal flora on fish and fish products; they get into the products through cross-contamination.

3.3.4.2 *Kayabo* sampling, transportation and storage

Systematic random sampling was used to pick samples (whole Kayabo) from the processors. Samples were directly purchased from randomly selected *Kayabo* processors from the two locations. A total of 30 samples were collected in sterile sampling bags, properly labelled and then placed into a sterile container (cool box). Samples were kept in a cool box maintained at < 5 ^{0}C and transported to the accredited National Fish Quality Control Laboratory (NFQCL), Mwanza for analysis. The samples that were not analysed on the same day were stored at -18^{0}C.

3.3.4.3 Analytical sample preparations and analytical methods

Analytical samples were prepared according to Andrew and Hammac (US - FDA BAM, 2001). For the enumeration of total viable counts (TVC), total coliforms, *E. coli* and *S. aureus*; 25 g of each *Kayabo* sample was weighed by using an analytical balance (Adventurer ^{TM} PRO OHAUS), then 225 ml of buffered peptone water (BPW) was added. The mixture was aseptically poured into a sterile stomacher bag and then blended / mixed by the stomacher (Seward STOMACHER ^{R} 3500 Lab System) for 1 minute to homogenize. For the detection of *L. monocytogenes*; 225 ml of Half Fraser broth for the primary enrichment were added to 25 g of the sample. The mixture was aseptically

poured into sterile stomacher bag and then mixed / blended by using the stomacher (Seward STOMACHER ^{R} 3500 Lab System) for 1 minute to homogenize. Then, the homogenate was incubated at 30 ± 1^{0}C for 24 ± 2 hours.

Enumerations of TVC, total coliforms, *E. coli* and *S. aureus* were respectively performed according to ISO 4833:2003 (ISO, 2003), ISO 4831:2006 (ISO, 2006), ISO 7251: 2005 (ISO, 2005), ISO 6888 – 3: 2003 (ISO, 2003), whereas detection of *L. monocytogenes* was done according to ISO 11290-1:1996/Amd 1: 2004 (ISO, 2004) methods. Reagents (diluents) and media used for the analyses were prepared according to the manufacturer's instruction(s) and specific test method requirements.

3.3.4.4 Microbiological results interpretation

The criteria used to judge the results are indicated in Table 3.1. Since fishery products from Lake Victoria (i.e. *Kayabo*) are domestically, regional (EAC) and internationally marketed; Tanzanian standards (TZS), East African Standards (EAS) and Codex Alimentarius Commission (CAC) standards were used to interpret the microbiological results.

Table 3.1: Microbiological criteria of fish and fishery products

S/N	PARAMETER	CRITERIA (Maximum Limit)	SOURCE
1	Total Viable Count (TVC)	1×10^{5} CFU/g	TZS 118:2007 EAS 828:2016
2	*E.coli*	1×10^{1} MPN/g Absent	TZS 731:2007 EAS 828:2016 CAC/RCP 52-2003
3	Total coliforms	4×10^{2} MPN/g	TZS 119:2002
4	*S. aureus*	1×10^{3} CFU/g 2×10^{3} CFU/g	TZS 125:2002 EAS 828:2016
5	*L. Monocytogenes*	Absent in 25g	CAC/RCP 52

3.3.4.5 Statistical data analysis

A hierarchical cluster analysis SPSS (Version 16.0 for Windows, SPSS Inc., Chicago, IL, USA) with the furthest neighbour method and squared Euclidean distance was used to analyze hygienic practices data. Non-parametric test (Kruskal Wallis Non Parametric H test) was performed to determine the differences among the clusters on the handling practices of *Kayabo*.

Microbiological quality data were analyzed by using the R statistical package (R Development Core Team, Version 3.0, Vienna – Austria). One way ANOVA was computed to determine the significant differences between the main factors. Means were separated by Duncan Multiple Range Test (DMRT). The statistical significance was established at $P<0.05$ level.

3.4 Results and Discussion

3.4.1 Handling practices of *Kayabo* along the processing chain

3.4.1.1 Hierarchical cluster analysis

Figure 3.1 shows clusters obtained by hierarchical cluster analysis (i.e. average linkage). Three clusters were formulated; cluster I (having 15 companies), cluster II (10 companies) and cluster III (5 companies). All cluster I processors were from Kanyama, whereas cluster II and III contained processors from Mwaloni. Processors from Kanyama were predominantly micro-scale and unregistered by neither TFDA, Municipal Council, nor Fisheries Department. In addition, their processing structures were constructed by non-permanent materials, not having a sound surface drainage system, no cooling (icing) during transportation of raw Nile perch from suppliers. Furthermore, inspections by Government officials were rarely carried out. Majority of cluster I companies obtain their raw materials as fish rejects from large fish processing companies.

The major differences between cluster II and III processors were on the production quantity and size of the company, however; both clusters had registered processors, sound drainage system and used ice during transportation of raw Nile perch from suppliers. However, in all clusters the processors were operating in an unhygienic condition without protective gears, personnel medical examination, food grade processing equipment, no cold storage of raw materials, poor packaging and inadequate storage condition and sanitation. All clusters were accessible by road and water used for processing was supplied by the Municipality.

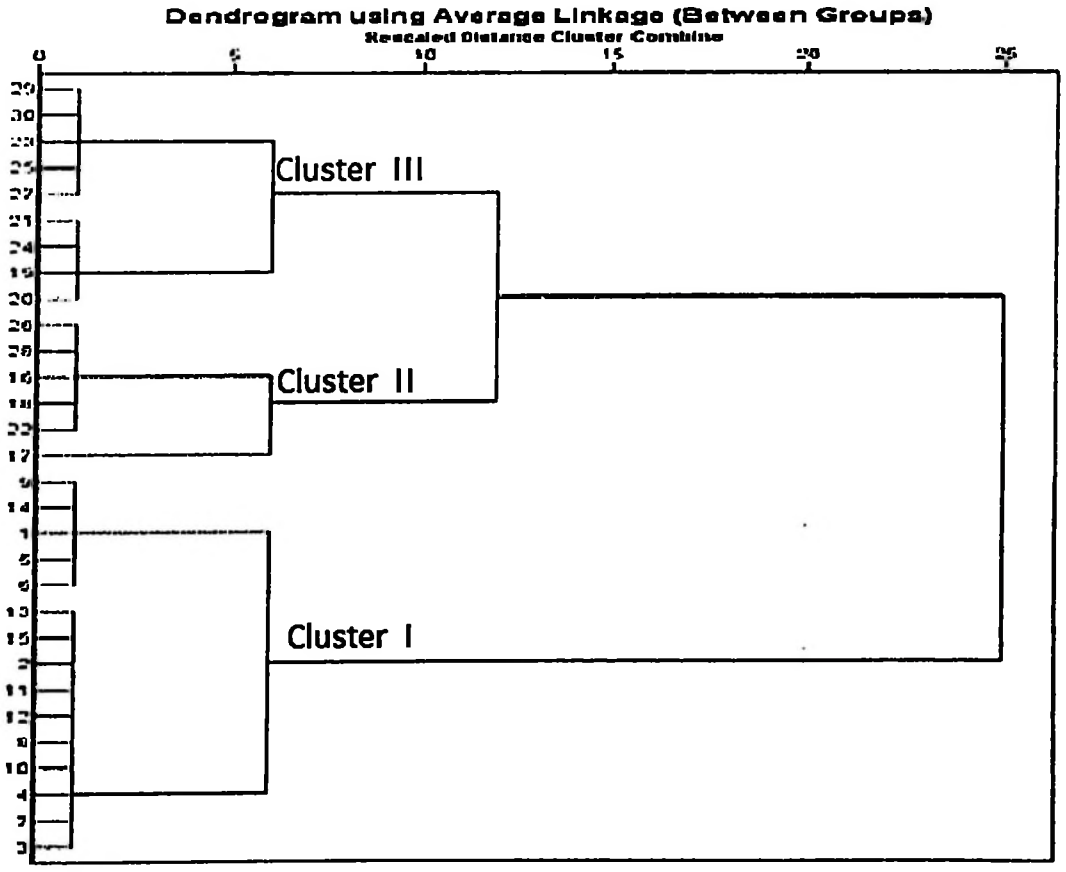

Figure 3.1: A dendrogram showing the clusters by hierarchical cluster analysis on the relationship of *Kayabo* processors in Kanyama and Mwaloni according to their handling practices

3.4.1.2 Characteristics of the *Kayabo* processors

Table 3.2 indicates that *Kayabo* processing is predominantly done by women processors 20 (66.67%). These processors are at micro (36.67%) and small (63.33%) scale level with weekly processing capacity of 1- 4 tons. Similarly, previous studies found that majority of women were engaged in fish processing activities such as fish smoking, salting and drying (Kweka *et al.*, 2006, Akande and Diei-Ouadi, 2010). Although none of the processors attended any special training on food hygiene and processing, all had primary school education (Table 3.2). This situation could result into inadequate processing and handling of *Kayabo*. Likewise, other studies reported low levels of formal education among the fishers and processors (Omwega *et al.*, 2006; Esser *et al.*, 2007; LVFO, 2008; Olale *et al.*, 2010). Well educated and trained personnel could use appropriate fish handling practices in value chain processes (Davies, 2009; Akande and Diei-Ouadi, 2010) which might reduce post-harvest losses and improve quality of the final product (*Kayabo*).

Table 3.2: Characteristics of the *Kayabo* processors (n=30)

Parameter	Category	Frequency (Percentage)		
		Cluster I	Cluster II	Cluster III
Gender	Male	5 (33.3)	3 (30.0)	2 (40.0)
	Female	10 (67.7)	7 (70.0)	3 (60.0)
	Total	15 (100)	10 (100)	5 (100)
Education level	Informal	0 (0)	0 (0)	0 (0)
	Primary	15 (100)	15 (100)	5 (100)
	Secondary	0 (0)	0 (0)	0 (0)
	Total	15 (100)	10 (100)	5 (100)
Number of employees	<10 (micro)	6 (40.0)	2 (20.0)	3 (60.0)
	10-49 (small)	9 (60.0)	8 (80.0)	2 (40.0)
	50-100 (medium)	0 (0)	0 (0)	0 (0)
	Total	15 (100)	10 (100)	5 (100)
Production volume	1 tons/week	15 (100)	0(0)	5 (100)
	2 tons/week	0 (0)	0 (0)	0 (0)
	3 tons/week	0 (0)	0 (0)	0 (0)
	4 tons/week	0 (0)	10 (0)	0 (00
	Total	15 (100)	10 (100)	5 (100)
Registration status	Not registered	15 (100)	0(0)	0 (0)
	Registered	0 (0)	10 (100)	5 (100)
	In process	0 (0)	0 (0)	0 (0)
	Total	15 (100)	10 (100)	5 (100)

n = frequency of respondents (*Kayabo* processors)

All clusters I and III processors had a weekly processing capacity of 1 ton, whereas cluster II companies could process 4 tons/week (Table 3.2). Cluster I processors operate in unregistered premises, poorly designed and unapproved facilities and receive no control / monitoring from the food safety control authorities or health officers from the Municipal. The products from such premises could be of poor quality associated with various food safety hazards that might endanger the public health (FAO / CDR, 2013).

3.4.1.3 Site, building layout and sources of water

Processors from the three clusters were operating in inadequately designed sites often without good drainage system that could result into cross contamination. Companies in cluster I had processing site which is not fenced to control pests, animals and un

authorized people into the facility. Animals were seen roaming around the processing site (cluster I) which could result into cross contamination. Although cluster II and III companies had fenced premises, there was no control of food vendors into the site. Fruits and vegetable vendors were selling their products in the site. However, *Kayabo* processing companies in all clusters (100%) were accessible by roads (Table 3.3). The buildings and equipment design and layout were not adequate; for instance, the buildings for cluster I (100%), cluster II (30%) and cluster III (80%) processors were not constructed with permanent materials. These buildings were constructed with wooden materials, roofed / thatched with plastic sheets and dried grass difficult to clean and with possibility of leakage during rains. It was further observed that, all processors from each cluster had no cold storage facilities and used non-food grade hygienically designed equipment. However, companies in all clusters used potable water from Municipal supply (Table 3.3). Lack of adequate knowledge on proper food handling accompanied by poor design and layout of the premises (i.e. no separation between dirty and clean processes) indicate that cross contamination was inevitable.

Table 3.3: Frequency distribution of respondents in Mwaloni and Kanyama according to their site, building layout and sources of water

Parameter		Category	Frequency (Percentage)		
			Cluster I	Cluster II	Cluster III
Site/Location	Free from contamination	Yes	0 (0)	0 (0)	0 (0)
		No	15 (100)	10 (100)	5 (100)
		Total	15 (100)	10 (100)	5 (100)
	Sound water drainage	Yes	0 (0)	10 (100)	5 (100)
		No	15 (100)	0 (0)	0 (0)
		Total	15(100)	10 (100)	5 (100)
	Road accessibility	Yes	15 (100)	10 (100)	5 (100)
		No	0 (0)	0 (0)	0 (0)
		Total	15(100)	10(100)	5 (100)
Building Layout /Equipment design	Adequate area / space	Yes	0 (0)	0 (0)	0 (0)
		No	15 (100)	10 (100)	5 (100)
		Total	15 (100)	10 (100)	5 (100)
	Permanent materials	Yes	0 (0)	7 (70.0)	1 (20.0)
		No	15 (100)	3 (30.0)	4 (80.0)
		Total	15 (100)	10 (100)	5 (100)
	Roofing	Yes	0 (0)	7 (70.0)	1 (20.0)
		No	15 (100)	3 (30.0)	4 (80.0)
		Total	15 (100)	10 (100)	5 (100)
	Food grade equipment	Yes	0 (0)	0 (0)	0 (0)
		No	15 (100)	10 (100)	5 (100)
		Total	15 (100)	10 (100)	5 (100)
	Cold storage	Yes	0 (0)	0 (0)	0 (0)
		No	15 (100)	15 (100)	5 (100)
		Total	15 (100)	10 (100)	5 (100)
	Cross contamination	Yes	15(100)	10(100)	5 (100)
		No	0(0)	0(0)	0 (0)
		Total	15 (100)	10 (100)	5 (100)
Water source	Municipal	Yes	15 (100)	10 (100)	5 (100)
		No	0 (0)	0 (0)	0 (0)
		Total	15 (100)	10 (100)	5 (100)

3.4.1.4 Sanitation and personnel hygiene

Table 3.4 shows that in all clusters, personnel were not subjected to any medical examination as recommended by the law. According to Tanzanian regulation, all food handlers need to check their health after every six months (TFDCA, 2003). Although section 45 of TFDCA, 2003 requires that every person in direct contact with food in food

processing and handling operations suffering from a septic sore, diarrhea, chronic cough or septic sore throat, typhoid, paratyphoid fever, any salmonella infection, dysentery or any staphylococcal infection liable to cause food poisoning; shall not be allowed to handle food and shall be required to seek medical attention for treatment; however, most of the processors do not adhere to it. It was also observed that, wounded personnel were handling food products without any protective gears.

All processors had no hygienically designed toilets and did not have hand washing points; cluster I processors had toilets (pit latrines) without water supply. Food handlers have never received any training on food processing and good practices (GHP and GMP) as a consequence, no body puts on any personal protective equipment and uniforms to minimize contamination. The observed inadequate operations could result into contamination with pathogenic and spoilage microorganisms of processing equipment and product. FAO/WHO (2013) reported that; majority of food-borne outbreaks occur as a result of failure of the food preparation procedures to adhere with good hygienic practices.

Fish is perishable product which needs good hygienic practices along the value chain to control microbial contamination and spoilage (Okonkwo *et al.*, 1993; Nguyen *et al.*, 2007). Inadequate cleaning and sanitation of processing equipment (containers, knives, contact surfaces, etc.) is a potential source of bacterial contamination in fish processing operations (Reij and Aantrekker, 2004; FAO/WHO, 2009).

Table 3.4: Frequency distribution of respondents in Mwaloni and Kanyama according to sanitation and personnel hygiene practices

Requirement		Category	Frequency (percentage)		
			Cluster I	Cluster II	Cluster III
Medical examination		Yes	0 (0)	0 (0)	0 (0)
		No	15 (100)	10 (100)	5 (100)
		Total	**15 (100)**	**10 (100)**	**5 (100)**
Personal hygiene facilities					
	Toilets	Yes	15 (100)	10 (100)	5 (100)
		No	0 (0)	0 (0)	0 (0)
		Total	**15 (100)**	**10 (100)**	**5 (100)**
	Adequacy of toilets	Yes	0 (0)	0 (0)	0 (0)
		No	15 (100)	10 (100)	5 (1000
		Total	**15 (100)**	**10 (100)**	**5 (100)**
	Availability of water at the toilets	Yes	0 (0)	10 (100)	5 (100)
		No	15 (100)	0 (0)	0 (0)
		Total	**15 (100)**	**10 (100)**	**5 (100)**
Protective gears		Yes	0 (0)	0 (0)	0 (0)
		No	15 (100)	10 (100)	5 (100)
		Total	**15 (100)**	**10 (100)**	**5 (100)**
Hygiene education		Yes	0 (0)	0 (0)	0 (0)
		No	15 (100)	10 (100)	5 (100)
		Total	**15 (100)**	**10 (100)**	**5 (100)**
Pre and post processing cleaning		Yes	0 (0)	0 (0)	0 (0)
		No	15 (100)	10 (100)	5 (100)
		Total	**15(100)**	**10 (100)**	**5 (100)**

3.4.1.5 Source of raw materials, processing and packaging of final products

Table 3.5 shows that, majority of *Kayabo* processors (80% cluster II, 67% cluster I and 60% cluster III) obtained their raw material (*Lates niloticus*) as rejects from large fish processing factories. Although the raw materials would require cold storage (between -1 and 4^0C) to prevent growth of micro-organisms and spoilage (Berkel *et al.*, 2004; Ghaly *et al.*, 2010), none of the processors had cold storage facilities. Prior to processing, washing of fresh Nile perch was done by all processors and fish curing was done by

using iodated salt. Rough food contact surfaces and drying racks made of wooden materials which are difficult to clean and sanitize were observed (Table 3.5).

Packaging of final products was done in non-food grade materials by using re-used, un-cleaned and sanitized polyethylene bags with approximately 50 kg per pack (bulky packaging). Moreover, there were no any labeling information (product name, origin, ingredients, production date, storage condition and batch number) on the pack of the product for all processors (Table 3.5). This might impede the product traceability and recall plan.

In addition, packaging of *Kayabo* was done in unhygienic condition as personnel were compressing the product by using bare feet. This condition pre exposes the products to Staphylococcal contamination as their natural reservoir is human skin, hair and superficial mucous membranes. Their presence in large numbers indicates the possible presence of enterotoxin as well as fault in sanitary or production practices (FAO/WHO, 2009; FAO / CDR, 2013). To prevent product contamination with spoilage and pathogenic micro-organisms, packaging should be done in hygienic condition (FAO/WHO, 2009). In addition, the packaging material should provide protection of the product from damage, blackening and rancidity (Ssebisubi, 2011). The dried fish should then be stored in a cool and dry environment (Nguvava, 2013).

Table 3.5: Frequency distribution (and percentages) of respondents in Kanyama and Mwaloni according to source of raw materials, processing and packaging of the final products (n=30)

Parameter		Category	Cluster I	Cluster II	Cluster III
Raw materials (Nile perch)	Specified sources	Rejects from fish factories	10 (66.7)	8 (80.0)	3 (60.0)
		Artisanal fishermen	2 (13.3)	1 (10.0)	2 (40.0)
		Rejects from landing sites	3 (20.0)	1 (10.0)	0 (0)
		Total	**15 (100)**	**10 (100)**	**5 (100)**
	Cold storage from the source	Yes	0 (0)	8 (80.0)	4 (80.0)
		No	15 (100)	2 (20.0)	1 (20.0)
		Total	**15 (100)**	**10 (100)**	**5 (100)**
	On site cold storage	Yes	0 (0)	0 (0)	0 (0)
		No	15 (100)	10 (100)	5 (100)
		Total	**15 (100)**	**10 (100)**	**5 (100)**
	Raw fish washing prior to processing	Yes	15 (100)	10 (100)	5 (100)
		No	0 (0)	0 (0)	0 (0)
		Total	**15 (100)**	**10 (100)**	**5 (100)**
	Type of salt used	Iodated	15(100)	10 (100)	5 (100)
		Rocky	0(0)	0 (0)	0 (0)
		Total	**15 (100)**	**10 (100)**	**5 (100)**
Processing	Food grade Equipment	In place	0 (0)	0 (0)	0 (0)
		Not in place	15 (100)	10 (100)	5 (100)
		Total	**15 (100)**	**10 (100)**	**5 (100)**
	Product packaging	Yes	10 (66.7)	7 (70.0)	4 (80)
		No	5 (33.3)	3 (30.0)	1 (20)
		Total	**15 (100)**	**10 (100)**	**5 (100)**
	Type of packaging	Food grade	0 (0)	0 (0)	0 (0)
		Non-food grade	15 (100)	15 (100)	5 (100)
		Total	**15 (100)**	**10 (100)**	**5 (100)**
	Labeling	Yes	0 (0)	0 (0)	0 (0)
		No	15 (100)	10 (100)	5 (100)
		Total	**15 (100)**	**10 (100)**	**5 (100)**

3.4.2 Microbiological quality of *Kayabo*

Total viable counts were quantified in all clusters with values ranging from 2.08 - 8.68 Log CFU/g (Table 3.6). Cluster I had 12 (80%) processors exceeding the maximum allowable limit (5 Log CFU/g) of TVC. *Staphylococcus aureus* were quantified in 12 (80%) companies (with 5 companies exceeding the allowable limit of 3 Log CFU/g) in cluster I, 1 (10%) companies in cluster II and 1 (20%) companies in cluster III. The rest of companies in all clusters were below the quantification limit (1 Log CFU/g). However, total coliforms were occasionally quantified in cluster I (2/15 companies) and cluster II (1/10 companies). *E. coli* and *L. monocytogenes* were not detected (A, P/25g) in all samples tested from all 30 processors.

The large counts beyond the acceptable limit for TVC ranging from 5.14 - 8.68 Log CFU/g and *S. aureus* ranging from 3.08 - 5.49 Log CFU/g of the analyzed samples from majority of processors in cluster I might be attributed to the observed unhygienic handling of raw materials and finished products, poor sanitation and personal hygiene, the use of non-food grade equipment (wooden materials as drying racks which are difficult to clean and sanitize hence harboring microorganisms) and inadequate packaging. According to literature, possible causes of food contamination by enteric microorganisms (*Enterobacteriaceae*) are inadequate procedures of handling, packaging and storage, sanitation and personal hygiene (Norman, 1999; Kussaga, 2015).

Table 3.6: Microbiological quality of *Kayabo* from cluster I, II and III

Cluster	Processors	TVC Log (CFU/g)	S. aureus Log (CFU/g)	Total coliforms Log (MPN/g)
I	K01	8.68^{a}	2.61^{i}	**
	K02	5.41^{l}	3.26^{e}	**
	K03	4.11^{n}	3.08^{g}	0.60^{b}
	K04	6.20^{f}	3.43^{d}	1.36^{a}
	K05	5.14^{k}	2.36^{k}	**
	K06	6.78^{e}	3.18^{f}	**
	K07	7.40^{d}	*	**
	K08	5.20^{j}	2.56^{j}	**
	K09	7.86^{b}	5.08^{b}	**
	K10	7.61^{c}	5.49^{a}	**
	K11	7.40^{d}	4.28^{c}	**
	K12	5.48^{h}	*	**
	K13	4.90^{l}	2.36^{k}	**
	K14	4.54^{m}	*	**
	K15	5.94^{g}	2.86^{h}	**
II	M01	3.65c	*	**
	M02	2.85g	*	**
	M03	3.20^{f}	*	**
	M04	3.51^{d}	*	0.60
	M05	4.82^{a}	2.65	**
	M06	3.81^{b}	*	**
	M07	2.11^{i}	*	**
	M09	2.34^{h}	*	**
	M11	2.08^{j}	*	**
	M13	3.41^{e}	*	**
III	M08	2.59^{d}	*	**
	M10	4.63^{a}	*	**
	M12	3.66^{b}	*	**
	M14	3.26^{c}	2.36	**
	M15	2.18^{e}	*	**
MAL		5	3	2

MAL means Maximum Allowable Limit (ISO; 4833:2003, 6888 – 3: 2003, 4831:2006, respectively). Letter K stands for Kanyama and letter M for Mwaloni
*** indicate microbial count < 1.0×10^{1} log (CFU/g), ** indicate microbial count <0.3×10^{1} log (MPN/g)**
Means with different superscripts within the same column are significantly different at *P<0.05*

An interesting part of the study was the compliance of the tested *Kayabo* samples from all clusters for total coliforms, *E. coli* and *L. monocytogenes* despite of the unhygienic practices of processors. This could be associated with the effect of salt (Sodium chloride)

on a_w as well as moisture content of *Kayabo* which inhibits the growth and proliferation of pathogenic microorganisms. Wijnker *et al.* (2006) studied antimicrobial properties of salt (NaCl) for the preservation of fish, fishery products and natural sheep casings at different water activity (a_w) levels and found the activities of most spoilage and pathogenic bacteria (*E. coli*, *Salmonella typhimurium*, *L. monocytogenes*, *S. aureus* and *E. coli* O157:H7) stopped when a_w of 0.89 was reached. The inhibitory effects of sucrose and sodium chloride on *S. aureus* are primarily related to a lower water activity (Dave and Ghaly, 2011). Salting decreases water activity and has inhibition effect on pathogenic bacteria (Teklemariam *et al.*, 2015; Yam *et al.*, 2015).

Although TVC, total coliforms and *S. aureus* were quantified in all clusters, only TVC indicated significant difference ($P<0.05$) among the clusters. Post Hoc (Bonferroni) test indicated significant difference ($P<0.05$) in TVC between clusters I and II and clusters I and III (Table 3.7). Cluster I had significantly high mean value of TVC (6.17 CFU/g), *S. aureus* (3.38 CFU/g) and total coliforms (0.98 MPN/g) respectively.

Table 3.7: Mean values of TVC, Total coliforms and *S. aureus* from cluster I, II and III

Parameter	Mean values		
	Cluster I	Cluster II	Cluster III
TVC	6.17^a	3.18^b	3.26^b
S. aureus	3.38^a	2.65^a	2.36^a
Total coliforms	0.98^a	0.60^a	0.00^a

Means with different superscripts on the same row are significantly different at $P<0.05$

3.5 Conclusions

The overall objective of this study was to assess the influence of the currently handling practices of *Kayabo* a salted sun-dried *Lates niloticus* from Lake Victoria, and its effect on the microbiological quality of the final product (*Kayabo*). The study showed that,

Kayabo processing chain was associated with poor quality raw material, unhygienic handling practices, lack of food grade processing equipment, poor sanitation, lack of hygiene training and education as well as poor packaging and storage of the products. These factors negatively affect the microbiological quality of the product which could contribute to post-harvest losses and food borne diseases.

Although, the processors receive inadequately handled raw materials (as rejects) from fish processing companies accompanied by very poor processing and storage conditions, some processors had products of better quality. If these processors are trained on best handling practices and receive quality raw materials, products of good microbial quality could be processed. The authors however, recommend further study on total mycotoxins in *Kayabo* taking into consideration the nature of the products and handling practices along the product value chain.

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CHAPTER FOUR

PAPER TWO

4.0 CHEMICAL QUALITY OF *KAYABO* - A SALTED SUN-DRIED NILE PERCH *Lates niloticus* FROM LAKE VICTORIA, TANZANIA

Nuru E. Mwasulama[1], Jamal B. Kussaga[2], Bendantunguka P. M. Tiisekwa[2]

[1]Tanzania Food and Drugs Authority, P. O. Box 77150, Dar es Salaam, Tanzania
E-mail: nurumwasulama@gmail.com

[2]Department of Food Technology, Nutrition and Consumer Sciences, Sokoine University of Agriculture, P. O. Box 3006, Morogoro, Tanzania
E-mail of the corresponding author: kussaga@suanet.ac.tz

[2]Department of Food Technology, Nutrition and Consumer Sciences, Sokoine University of Agriculture, P. O. Box 3006, Morogoro, Tanzania
E-mail: tiisekwa@suanet.ac.tz

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4.1 Abstract

The chemical quality of a salted sun-dried *Lates niloticus* (*Kayabo*) from Lake Victoria was investigated. Thirty samples of *Kayabo* were collected from thirty processors from two Municipalities of Mwanza City namely Nyamagana (Kanyama) and Ilemela (Mwaloni). Samples were analyzed for moisture content and fish spoilage indicators TVB-N and TMA-N. The analyzed samples from the two locations varied significantly ($P<0.05$) in moisture content (MC), TVB-N and TMA-N contents. Samples from Mwaloni had significantly higher mean scores of moisture content (42.8%) compared to Kanyama (27.4%). The MC of *Kayabo* from Mwaloni exceeded the 30% cut-off point for dried-salted fishery products. About 60% of *Kayabo* samples from Kanyama had

TVB-N concentration beyond the freshness scale (between 20 – 30 mg N/100 g) whereas, 33 % of samples from Kanyama exceeded the TMA-N acceptable level (> 5 mg TMA-N/100g). All samples from Mwaloni had TMA-N and TVB-N values within the acceptable limit. The higher concentration of TVB-N and TMA-N beyond the freshness scale from majority of *Kayabo* samples from Kanyama indicated use of raw materials which had started to spoil. Therefore, control measures such as improving the handling practices by using cold storage from the source of raw materials to the processing units, strict control on receipt of raw materials (fish and salt), use of potable water, improving hygiene and sanitation of the facility, proper drying, appropriate packaging and proper storage of the product are recommended.

KEY WORDS: fish, Nile perch, salted sundried Nile perch, *Kayabo*, chemical quality (Moisture content, TMA-N, TVB-N), Lake Victoria, specific spoilage organisms.

4.2 Introduction

Lake Victoria is the world's second largest fresh water body and the largest in Africa, it occupies a surface area of 68 800 km^2 and catchment area of 284 000 km^2 (Josupeit, 2006; Mhongole, 2009). It is a relatively shallow Lake with an average depth of 40 metres and a maximum depth of approximately 80 metres. The lake is shared by three riparian countries; Tanzania (49%), Uganda (45%) and Kenya (6%) (Josupeit, 2006; FAO, 2007; Mhongole, 2009). The fisheries of Lake Victoria provide an immense source of income, employment, food and foreign exchange to the riparian states (LVFO, 2008; URT, 2013).

Fish is one of the most highly perishable food products; unless well handled, fish quality deteriorates so rapidly limiting the shelf life (Quang, 2005; Matís, 2010; Dergal *et al.*,

2013). Once the fish dies, several postmortem changes take place due to the breakdown of the cellular structures and growth of microorganisms (Quang, 2005; El-Sherif and Abd El-Ghafour, 2016). The postmortem changes affect quality and shelf life as are associated with protein and ATP degradation, pH drop, lipid oxidation, undesirable compounds production such as trimethylamine (TMA-N) and low molecular weight volatile bases (TVB-N) (Ghaly *et al.*, 2010; Fathia *et al.*, 2013; Teklemariam *et al.*, 2015).

Recent studies on fish and fishery products indicated that quality and safety of fish and fishery products can be ascribed to their total volatile basic nitrogen (TVB-N), trimethylamine (TMA) and moisture content. Total volatile basic nitrogen is important characteristic for the assessment of quality in seafood products and appears as the most common chemical indicators of marine fish spoilage (Wu and Bechtel, 2008; Amegovu *et al.*, 2012). It is commonly used as an estimate of spoilage and has been widely used as an index for freshness (Wu and Bechtel, 2008; Jinadasa *et al.*, 2014). The pungent odour of spoiled fish has been related to the TMA-N tissue levels but also with the number of spoilage organisms present in many fish species (Fathia *et al.*, 2013). It is often used as an index in evaluating the shelf-life and keeping quality of fish products (Fathia *et al.*, 2013). There is a close relationship between the moisture content and bacterial load in food products (Ghaly *et al.*, 2010; Dergal *et al.*, 2013). Lowering the moisture content in fish minimizes putrefaction and improves its keeping quality (Abbas *et al.*, 2009; Ghaly *et al.*, 2010; Dergal *et al.*, 2013).

In Tanzania, majority of marketed fishery products are from small scale processors (Kabahenda, 2009; Kussaga, 2015; Teklemariam *et al.*, 2015). These artisans employ various traditional methods including smoking, drying, salting, frying, fermenting and combinations of these (hurdles) to preserve and process fish. Salted sun dried *Lates*

niloticus commonly known as *Kayabo* is among the major product processed by these small processors for domestic and regional markets i.e. East African Common Market (EACM) and Common Market for Eastern and Southern Africa, COMESA (Abila and Konstantine, 2006; Josupeit, 2006; Kabahenda *et al.*, 2011). Furthermore, majority of artisans in African countries including Tanzania are faced with lack of cold chain, good infrastructure and adequate facilities for transportation of fresh fish from the sources to the processors (FAO, 2014). These constraints affect quality of raw fish resulting into poor quality fishery products (Quang, 2005; Kabahenda, 2009; FAO, 2014; Getu *et al.*, 2015).

Fish products from small-scale processors are susceptible to rejection due to poor quality (FAO / WHO, 2004; Kussaga, 2015; Teklemariam *et al.*, 2015). To date, there is no documented study in Tanzania on the chemical quality of *Kayabo*. This study therefore, seeks to investigate the chemical quality of *Kayabo*. The information generated from this study would be useful to fish processors, researchers, food control authorities and policy makers to set policies and strategies to monitor and control small-scale processors to manufacture products of good quality that would meet domestic and export markets standards.

4.3 Materials and Methods

4.3.1 Chemical assessment

4.3.1.1 Selection of chemical parameters

The freshness and spoilage indicators of fishery products, trimethylamine nitrogen (TMA-N) and total volatile base nitrogen (TVB-N) as well as food products keeping quality indicator (moisture content) were selected (Collins, 1980; EC, 2005; Ozogul *et al.*, 2005; Zhou *et al.*, 2011; Amegovu *et al.*, 2012; Immaculate *et al.*, 2013; Dergal *et al.*, 2013; Fathia *et al.*, 2013; Jinadasa *et al.*, 2014). These parameters are universally

used as the criteria for the fitness for consumption and keeping quality of fish and fishery products (Dergal *et al.*, 2013). The TVB-N measurements indicate the extent of the breakdown of protein due to bacterial and enzymatic actions leading to amine production (Immaculate *et al.*, 2013). Enzymes from the spoilage microorganisms can metabolize amino acids of the fish muscle to produce tri-methylamine, di-methylamine and ammonia.

4.3.1.2 Sampling, transportation and storage of samples

The salted sun dried Nile perch were directly purchased from the selected *Kayabo* processors from the two locations (Kanyama and Mwaloni). A total of 30 samples from 30 small-scale processors were taken and inserted in well labelled sterile sampling bags, and then placed into a sterile cool box. Samples were transported in a cool box maintained at < 5 ^{0}C to the National Fish Quality Control Laboratory (NFQCL). The samples were then stored at -18^{0}C waiting for analyses.

4.3.1.3 Analytical sample preparations and analyses

The TVB-N of *Kayabo* were extracted according to the European official method (EC) 2074/2005 (EC, 2005). A 100 g of the sample to be analyzed were taken by using scissor and ground by using a meat grinder (IKA A11 BASIC S2) to produce a homogeneous fish mince, then mixed with 90 ml of 0.6 M perchloric acid solution. The mixture was homogenized for 2 minutes and then filtered (Fluted filter with 150 mm diameter). The filtrate was distillated by steam distillation whereby the distillation outflow tube was submerged in a receiver (Erlenmeyer conical flask) containing 100 ml of 0.3 M boric acid solution to which three drops of Tashiro mixed indicator (2 g methyl-red and 1 g methylene-blue dissolved in 1 000 ml 95% ethanol) was added. The volatile bases contained in the distillate were determined by titration with a standard hydrochloric acid solution (0.05 N) and expressed as mg TVB-N per 100 g of the fish muscle. To assay

specifically nitrogen generated by the trimethylamine (TMA), distillation method was applied in presence of formaldehyde (to block the primary and secondary amines whilst leaving only the tertiary amines to react) and results were expressed as mg TMA-N per 100 g of the fish muscle. Moisture content was determined by AOAC method 985.14 (AOAC, 1995). The automatic moisture balance analyzer device (Moisture Balance Analyzer ADAM, AMB 310 United Kingdom) was used to dry 5 g of fish muscle at 105°C for two hours to a constant weight.

4.3.1.4 Results interpretation

European Union Community (EC) and East African Community Standards (EAS) were used to interpret the results (Table 4.1).

Table 4.1: The criteria for the interpretation of chemical quality of *Kapenta*

Chemical parameter	Requirement	Criteria	Standard / Reference
Moisture content (%)	<12	Adequately dried product (Shelf stable)	EAS 828 : 2016 (EAS, 2016) Kabahenda *et al.*, 2011
	12-30	Slighty microbial activities (immediate intervention required)	
	>30	Critical point. high microbial activities (Shelf limited product)	
TVB-N (mg N / 100 g muscle)	<20	Good quality (fresh) fish	(EC) 2074/2005 (EC, 2005) Fathia *et al.*, 2013
	20 - 30	Beginning of fish spoilage (stale)	El-Sherif and El-Ghafour, 2016 Turkish Manual of Seafood Quality Control, 2008.
	>30	Spoiled fish (un fit for human consumption)	
TMA-N (mg N / 100 g muscle)	<5	Good quality fish	EC) 2074/2005 (EC, 2005) Fathia *et al.*, 2013
	5 - 15	Rejection levels)	El-Sherif and El-Ghafour, 2016 Turkish Manual of Seafood Quality Control, 2008.

4.3.2 Statistical data analysis

Data for chemical quality of *Kayabo* were analyzed by using R statistical package (R Development Core Team, Version 3.0, Vienna – Austria). One way ANOVA was computed to determine the significant differences in TVB-N, TMA-N and moisture contents among the processors in both locations. Means were separated by Duncan's Multiple Range Test. Student t - test was used to test the significant difference for each parameter between the two locations. The statistical significance was established at $P<0.05$ level.

4.4 Results and Discussion

4.4.1 Chemical quality of *Kayabo*

4.4.1.1 Moisture content of *Kayabo* from processors in Kanyama and Mwaloni

Moisture content of *Kayabo* samples from the two locations (Kanyama and Mwaloni) varied significantly ($P<0.05$, Table 4.2). Samples from Mwaloni had higher mean moisture content (42.2%) compared to samples from Kanyama (27.4%). Out of 30 *Kayabo* samples analyzed, 21 (70%) samples from the two locations (Mwaloni 100% and Kanyama 60%) had the moisture content beyond the maximum allowable limit (30%). The moisture content of *Kayabo* obtained from this study was in the range of 12.1% (sample from Kanyama) to 50.0% (sample from Mwaloni). Samples from Kanyama (12.1 – 46.1%) indicated highest variability in moisture content than those from Mwaloni (38.4 – 50.0%) (Table 4.2).

Table 4.2: Moisture content (%) of *kayabo* from processors in Kanyama and Mwaloni

Processor	Location	
	Kanyama	Mwaloni
1	28.5^{f}	44.1^{c}
2	28.3^{f}	39.9^{de}
3	37.7^{d}	38.4^{e}
4	43.6^{b}	47.3^{b}
5	12.5^{j}	41.3^{d}
6	23.3^{h}	39.4^{de}
7	23.2^{h}	42.3^{d}
8	12.1^{j}	38.8^{e}
9	39^{c}	45.2^{bc}
10	46.1^{a}	45.9^{bc}
11	30^{e}	39.5^{de}
12	30.2^{e}	41.9^{d}
13	24.4^{g}	50.0^{a}
14	12.2^{j}	48.2^{ab}
15	20.4^{i}	39.9^{de}
$\bar{Y}$	27.4^{a}	42.2^{b}

Mean values with different superscript letters along the same column are significantly different at *P<0.05*
Overall means on the same row with different superscript are significantly different at *P<0.05*

The observed variation in moisture content of *Kayabo* could be attributed by the variation in drying time, environmental changes (as drying was done by direct sun-drying) as well as level and type of salt used for curing. Comparable results on the moisture content of salted sun-dried fish ranging from 12% to 50% were reported in studies by Kabahenda *et al.* (2011), Prakash *et al.* (2011), Ayinsa and Maalekuu (2013), Immaculate *et al.* (2013) and Tariqul *et al.* (2013). The results obtained from the present study revealed that, moisture content of majority of *Kayabo* was much higher than the recommended moisture content for the dried salted fish (>30%), thus making *Kayabo* susceptible to molds and other spoilage bacteria growth (Tariqul *et al.*, 2013). According to Prakash *et al.* (2011), and Immaculate *et al.* (2013), when moisture content of dried

salted fish fell below 30% of wet weight, bacterial action stopped and when the moisture content further reduced to 15%, mold ceased to grow. Kabahenda *et al.* (2011) reported that, generally no microorganism can grow in a dried salted fish with moisture content below 15%. Moisture content of seafood's plays an important role in spoilage and lowering of moisture retards the spoilage (Kabahenda *et al.*, 2011; Prakash *et al.*, 2011).

4.4.2.2 Total volatile basic nitrogen (TVB-N) content of *Kayabo*

The overall mean values of TVB-N content (mg N/100g) of *Kayabo* samples from Kanyama and Mwaloni varied significantly ($P<0.05$). The overall mean value of TVB-N content for samples from Kanyama was significantly ($P<0.05$) higher (18.2 mg N/100g) compared to that from Mwaloni (5.45 mg N/100g) (Table 4.3). In addition, a significant difference ($P<0.05$) in TVB-N values of the analyzed samples from different processors was observed between the locations. Samples from Kanyama indicated higher TVB-N values (5.5-24.2 mg N/100g) compared to those from Mwaloni, of all the samples analyzed, only two samples from Mwaloni had detectable TVB-N (5.4 – 5.5 mg N/100g) (Table 4.3). Compliance of Mwaloni samples with TVB-N might be attributed by the use of ice by majority of raw material suppliers.

Table 4.3: TVB-N (mg N/100 g) content of *Kayabo* from processors in Kanyama and Mwaloni

Processor	Location	
	Kanyama	Mwaloni
1	22.0^{b}	*
2	18.6^{c}	*
3	6.1^{d}	*
4	20.2^{b}	*
5	5.5^{e}	5.4^{b}
6	23.8^{a}	*
7	20.7^{bc}	*
8	5.9^{de}	*
9	24.2^{a}	*
10	23.2^{ab}	*
11	21.8^{b}	*
12	*	*
13	23.5^{a}	*
14	*	5.5^{a}
15	21.1^{bc}	*
$\bar{Y}$	18.2^{a}	5.45^{b}

Mean values with different superscript letters along the same column are significantly different at *P<0.05*
Overall means on the same row with different superscript are significantly different at *P<0.05*
*** indicates TVB-N concentration below 5mgN/100g (below the detection level)**

Majority of processors get their raw materials as rejects from industrial fish processing companies (Kabahenda, 2009). Majority of *Kayabo* samples (60%) from Kanyama had the TVB-N concentration beyond the freshness scale (between 20 – 30 mg N/100 g). This suggests that, *Kayabo* is made from spoiled raw materials (Jianadasa *et al.*, 2014). The level of TVB-N for white fish is generally considered fresh if the TVB-N value is less than 20 mg N/100g sample, beginning of spoilage when 20–30 mg N/100g and spoiled when over 30 mg N/100g (Egan *et al.*, 1981; Castro *et al.*, 2006; Turkish Manual of Seafood Quality Control, 2008; Fathia *et al.*, 2013; Immaculate *et al.*, 2013; Jinadasa *et al.*, 2014 ; El-Sherif, 2016).

The TVB-N production increases with increase of the holding time resulting into quality loss of fish in an un-frozen state (Quang, 2005; Sallam, 2007; Ghaly *et al.*, 2010;

Immaculate *et al.*, 2014; Jinadasa *et al.*, 2014). When the temperature decreases the bacterial growth is slower, the reaction rate of enzymes is also decreased and the rigor mortis time can be extended (Quang, 2005; Ghaly *et al.*, 2010). Once the fish raw material freshness and nutrition value is lost, it cannot be recovered in the processing stages (Quang, 2005). Rejects from fish processing factories, landing sites and artisanal fishers and inadequately handled (no cold chain) fish are used as raw materials to process *Kayabo* (Kabahenda, 2009; Ayinsa and Maalekuu, 2013).

Chemical results from this study correlate with microbiological quality of *Kayabo* reported by Jianadasa *et al.* (2014). The same samples with the highest TVC indicated high TVB-N content. Therefore, production of TVB-N levels in fish muscles correlates with bacterial growth (Immaculate *et al.*, 2013).

4.4.2.3 Trimethylamine nitrogen (TMA-N) content of *Kayabo*

The overall mean scores of TMA-N content of *Kayabo* between Kanyama and Mwaloni are presented in Table 4.4. The two locations varied significantly ($P<0.05$) in TMA-N content; processors from Kanyama had significantly higher overall mean scores ($\bar{Y} = 4.2$) of TMA-N than those from Mwaloni ($\bar{Y} = 1.9$).

The TMA-N values of *Kayabo* among the processors at Kanyama varied significantly ($P<0.05$), the values ranged from 1.6 – 7.0 mg N/100g (Table 4.4). A total of 33.3% of *Kayabo* samples from Kanyama had TMA-N values beyond the acceptable level (> 5 mg TMA-N/100g). All samples from all processors at Mwaloni had TMA-N values within the acceptable limit (< 5mg TMA-N/100 g of the fish muscle), suggesting that processors use good quality fish to process *Kayabo*.

Table 4.4: TMA content (mg N/100g) of *Kayabo* from processors in Kanyama and Mwaloni

Processor	Location	
	Kanyama	Mwaloni
1	5.4^{c}	**
2	4.0^{de}	**
3	3.0^{d}	**
4	4.1^{c}	**
5	1.6^{g}	2.2^{a}
6	3.6^{e}	**
7	4.4^{d}	**
8	1.7^{g}	**
9	7.0^{a}	**
10	5.8^{b}	**
11	5.5^{bc}	**
12	**	**
13	5.6^{bc}	**
14	**	1.6^{b}
15	2.4^{f}	**
$\bar{Y}$	4.2^{a}	1.9^{b}

Mean values with different superscript letters along the same column are significantly different at *P<0.05*
Overall means on the same row with different superscript are significantly different at *P<0.05*
**** indicate Not Detected**

Previous studies found that, fish rejected by sensory panels varied between fish species, but is typically having around 5 -15 mg TMA-N/100g for aerobically stored fish (El-Marrakch *et al.*, 1990; Riquixo, 1998; Fathia *et al.*, 2013; El-Sherif and Abd El-Ghafour, 2016). The compliance with TMA-N levels of *Kayabo* from Mwaloni might be attributed by the use of cold chain (ice) by majority of suppliers during the handling of the raw Nile perch from the source. Proper storage temperature is critical for maintaining fish and fishery products safety and quality (FAO / WHO, 2009; FAO, 2014). Quality of fish raw material plays an important role for the quality of the end-product.

Tri-methylamine originates from bacterial decomposition of fish (Immaculate *et al.*, 2013; El-Sherif and Abd El-Ghafour, 2016). The presence of TMA-N in fish is therefore, taken as an indication for bacterial growth, while the ammonia comes from decomposition of amino acids, thus reducing the quality of the available protein

(Jinadasa *et al.*, 2014). Huss (1988) reported that, formation of TMA-N in fish muscle is due to the increase of spoilage bacteria levels. Similar samples with higher TVC counts (Immaculate *et al.*, 2013) had also higher TMA-N, suggesting a significant positive correlation in microbiological level and TMA-N production. Previous studies reported a correlation in the formation of TMA-N in fish muscle (the pungent spoiled fish odour) with increase in spoilage bacteria (Huss 1988; Quang, 2005; Ghaly *et al.*, 2010; Fathia *et al.*, 2013; Immaculate *et al.*, 2013; Jinadasa *et al.*, 2014).

4.4.2.4 Correlation between TVB-N, TMA-N and moisture content of *Kayabo*

Table 4.5 indicates the correlation between TVB-N and TMA-N production, moisture content and TVB-N production as well as moisture content and TMA-N production. There was a strong correlation (0.973) between TVB-N and TMA-N production. However, a negative correlation was observed between moisture content and TVB-N production (-0.373) and moisture content to TMA-N production (- 0.307) (Table 4.5). These results correlate with the findings reported by Immaculate *et al.* (2013) and Jinadasa *et al.* (2014) that TMA-N production is depended on the quantifiable production of TVB-N in the fish muscle. TVB-N content increases parallel with the increase in TMA during spoilage; as the activity of spoilage bacteria increases after death of a fish, subsequent increase in the reduction of TMAO to TMA occurs (Yusuf *et al.*, 2010). Total volatile basic nitrogen (TVB-N) measures the content of trimethylamine (TMA), dimethylamine (DMA), ammonia and other basic nitrogenous compounds, is commonly used as an estimate of spoilage and has been widely used as an index for freshness of fish (Immaculate *et al.*, 2013; Jinadasa *et al.*, 2014).

Table 4.5: Correlation between TVB-N, TMA-N and moisture content of *Kayabo*

Parameter	Correlation (r)	P_{value}
TVB-N versus TMA-N	0.973	0.000
MC versus TVB-N	- 0.373	0.023
MC versus TMA-N	- 0.307	0.053

4.5 Conclusions

This study evaluated the chemical quality of salted sun-dried *Lates niloticus* (*Kayabo*) from Lake Victoria in Tanzania. High values of moisture content, TVB-N and TMA-N were recorded in majority of *Kayabo*, giving an indication of low quality product and high susceptibility to spoilage hence, reducing its keeping quality and rendering unfit for human consumption. This study concludes that, *Kayabo* has poor chemical quality as some of the quality parameters tested including moisture content, TVB-N and TMA-N were beyond the recommended limits. Therefore, control measures such as training on best handling practices, cold storage, raw material inspection, food grade processing equipment, use of effective drying methods, and proper packaging are recommended. In addition, proper monitoring of small scale processors is highly required to ensure processing of quality products.

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CHAPTER FIVE

5.0 GENERAL CONCLUSION AND RECOMMENDATION

5.1 Conclusions

Kayabo value chain is associated with poor quality raw material as a result of lack of cold storage during handling of raw fish throughout the processing chain, inadequate handling practices, lack of food grade processing equipment, poor sanitation, lack of hygiene training and education as well as poor packaging and storage of the product. The higher bacterial count (TVC), moisture content and higher concentration of TVB-N and TMA-N beyond the freshness scale from majority of *Kayabo* samples indicates that consumers are at risk of contracting food safety hazards. Proper control of raw materials and processing conditions would produce better quality *Kayabo.* Poor quality raw material produces poor quality food product that could result into food safety problems and endanger the public health.

5.2 Recommendations

Based on popularity of *Kayabo* among consumers, it is necessary to take actions that will improve the quality and safety status in order to protect the health of consumers. This study recommends that:

i) Provision of training to the *Kayabo* processors on the Good Hygienic Practices (GHP) and Good Manufacturing Practices (GMP) to ensure safe and quality product.

ii) Proper control of raw materials by developing supplier specifications to prevent poor quality raw materials from being accepted for processing.

iii) Routine inspection of processors by the food control authorities to ensure adequate processing and cold chain by the processors that will prevent spoilage and growth of spoilage and pathogenic microorganisms.

iv) Improved *Kayabo* processing method with specific salt concentration per unit mass, appropriate drying technique (s) as well as appropriate final product packaging, labeling and storage.

v) Further studies to analyze the total mycotoxins in *Kayabo* and shelf life.

APPENDIX

Appendix 1: Observation checklist

Date:.....

Name of processor/ Company .....

Sex: .....

Location / District.....

Item/ Aspect/ Requirement	In place	Not in place	Do not know
1. Site/Location			
a) Free from sources of contamination			
b) Sound surface water drainage in place			
c) Accessible by road			
2. Building/ Construction/ equipment			
a) Provide adequate area to accommodate activities carried on			
b) Of Permanent material and good state of repair			
c) Are equipment/ utensils for handling and processing of food grade? (smooth, non-absorbent, no accumulation of grime or dirt)			
d) Is there cold storage equipment in place?			
3. Source water			
• source is (Municipal or private) potable and available all the time • Direct from the lake • Direct from well • or any other source (mention);.....	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐
4. Raw materials (raw Nile perch)			
a) Raw materials from approved source? (Artisanal fishing, fish factories). Any other source of raw material (mention)..... b) Rejects from fish processing companies			
c) Is ice used to store raw fish from the suppliers prior receipt?			
d) Are raw fish stored properly and away from rodents, insects, and other contaminants?			
e) Are raw Nile perch washed prior processing?			

f) What is the source of salt/ brine used? • Approved shops/ wholesalers • Other source; (mention).....			
5. Sanitation & Personnel hygiene			
a) Employees/ processors medically examined at on first appointment/ and after six months			
b) Personal hygiene facilities in place? e.g. hands washing points			
c) Are protective gears and uniforms provided?			
d) No direct communication between clean(processing area) and dirty side (waste disposal / toilet(s))			
e) Are toilets able to accommodate the number of processors in place?			
f) Are the toilets equipped with water supply?			
g) Are the processors obtaining good hygienic practices education from Government departments?			
6. Inspection by Government Officials			
a) Are inspection(s) conducted to the site?			
7. Packaging and Storage			
a) Is the storage room well roofed with no leakages?			
b) Are the materials used for packaging of food grade and appropriately labelled?			
8. Quantity processed / day or year / week etc. (processing capacity)			
9. Education level			
10. Number of employees.....			
11. Registration status of the processor (TFDA, Municipal, Fisheries department)			