DEVELOPMENT AND VALIDATION OF INDICES OF QUALITY OF LAKE VICTORIA NILE PERCH (*Lates niloticus*) MARKETED IN TANZANIA

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ABSTRACT

This study was conducted to validate the Quality Index Method (QIM) scheme and determine storage days on ice for domestically processed Nile perch (*Lates niloticus*). Samples collected from the fishermen at landing site were used to develop the QIM and for shelf life study at 0ºC to -1ºC for 33 days. Those collected from fish factories and cold stores were used for validation. The study assessed the sensory, microbiological and chemical quality changes of Nile perch on ice. The QIM scheme had the maximum total demerit points score 23 and 24 for gutted and ungutted fish, respectively. The Total Viable Count (TVC) and Specific Spoilage Bacteria (SSB) at the end of storage time (33 days) were 7.64 and 7.36 $\log_{10}$ cfu/g for ungutted and 6.93 and 6.83 $\log_{10}$ cfu/g for the gutted fish, respectively**.** The TVC and SSB exceeded the limit of acceptability set by Tanzania standards, beyond 14 storage days. Total Volatile Basic Nitrogen (TVBN) value increased slowly with storage time, reaching 11.48 and 12.18 mgN/100g in day 33 of storage for gutted and ungutted fish, respectively. Peroxide values (PV) increased gradually for ungutted Nile perch up to 21 days whereas, rapid increase was observed in gutted Nile perch up to14 days. The Quality Index (QI) scores had a positive correlation (0.91 for TVC, 0.97 for SSB, 0.79 for TVBN) and negative correlation (- 0.40 for FFAs and - 0.33 for PV). There was a significant decrease ($p<0.05$) in quality of cooked Nile perch from day 14 onwards. The storage time for good freshness quality is 14 days on ice. For good sensory and microbiological quality, Nile perch should not be stored on ice for more than two weeks. Adoption of QIM to determine the freshness level and storage time of Nile perch is necessary.

DECLARATION

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

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DEDICATION

This research work is dedicated to my beloved parents, Mr. and Mrs. Felix G. Massawe, brothers and sisters for their prayers, encouragement and valuable assistance in completion of this work.

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%	Percentage
&	And
˚C	degree census
<	less than
>	greater than
∑	Summation
∑C	Total number of colonies
≤	Less than or equal to
ANOVA	Analysis of Variance
BPW	Buffered Peptone Water
Cfu	colony forming unit
CH_3CI	Chloroform
DMA	Dimethylamine
et al	and others
EU	European Union
FAO	Food and Agriculture Organization of United Nations
FeS	Ferrous sulphide
FETA	Fisheries Education and Training Agency
FFAs	Free Fatty Acids
Fig.	Figure
G	Gutted
G	Gram
H	Hour

H_2O_2	Hydrogen peroxide
H_2S	Hydrogen Sulphide
H_2SO_4	Sulphuric acids
HACCP	Hazard Analysis Critical Control Point
ICMSF	International Commission on Microbiological Specification for Food
ICS	International Classification for Standards
ISO	International Organization for Standardization
Kg	Kilogram
Log_{10}	logarithm base ten
Ltd	Limited
M	Molarity
mEq	Milliequivalent
Mg	Milligram
Min	Minutes
Ml	Millilitre
MOLFD	Ministry of Livestock and Fisheries Development
N	Normality/Nitrogen
$Na_2S_2O_3$	Sodium thiosulfate
NaOH	Sodium hydroxide
NFQCL	National Fish Quality Control Laboratory
PV	Peroxide Value
Pvt	Private
QI	Quality Index
QIM	Quality Index Method
S	Seconds

Spp	Species
SSB	Specific Spoilage Bacteria
TCA	Trichloroacetic acid
TMA	Trimethylamine
TMAO	Trimethylamine Oxide
TRAHESA	Training and Research for Aquatic Health in Eastern and Southern Africa
TVBN	Total Volatile Basic Nitrogen
TVC	Total Viable Counts
TZS	Tanzania Standards
UNG	Ungutted
URT	United Republic of Tanzania
UV	Ultra Violet
V	Volume
W	weight
w/v	weight by volume

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Nile perch (*Lates niloticus*) is among the most important commercial fish species from the Lake Victoria. It was introduced in the 1950s and it is the most valuable freshwater fishery in Africa. Since the 1990s has supported an extremely valuable export-orientated fishery that generates a significant source of revenue for the population of the three riparian countries. Nile perch is processed at lake-side plants and exported as chilled or frozen. It contributes to foreign currency earnings, in addition to the sector's important role in income generation, employment, food security and nutrition such as protein, vitamins, and minerals as well as omega-3 fatty acids, which is a key nutrient for brain development (Coletta *et al.,* 2010). The Nile perch processors are divided into two, those who process for export markets mainly to lucrative European Union (EU) countries. Others are small-scale processors which target domestic and regional markets. Fish that are selected for factory processing are those which are organoleptically (sensory) excellent and of very good quality (Karungi *et al.,* 2004; MOLFD, 2013). However, there is no inspection and monitoring of good hygienic fish handling and processing is applied for small scale processors who process for domestic market (Kyangwa and Odongkara, 2005).

Fish quality is a complex concept involving a whole range of factors, which for the consumer include for example nutritional quality, availability, convenience and integrity, freshness, eating quality and the obvious physical attributes of the species, size and product type. It may also involve safety aspects such as being free from harmful bacteria, parasites or chemicals. Nevertheless, freshness makes a major contribution to the quality of fish as are perishable products. From the moment the fish is caught, the deterioration

process starts and its quality as a food product is affected. Changes occur in composition and structure caused by biochemical, physical, enzymatic and bacterial reactions, that adversely upsetting the sensory quality of the fish product. These can be caused by poor handling, processing and storage techniques, including time/temperature (Martinsdóttir, 2002; Abbas *et al.,* 2008). Thus, in order to maintain the freshness of fish and avoid microbial contamination, the simplest and effective means of slowing down deterioration is by using ice in preserving catch throughout handling. It can preserve the fish up to 2-3 weeks which depends on fish species. When a fish dies, the bacteria that are on the skin and intestine proliferate and invade the flesh resulting in quality deterioration (Jorge *et al.,* 2016). It has been reported that the ice is not effective for long term storage because the flora of the fish such as *Pseudomonas spp*. and *Shewanella putrefaciens* can grow even when the fish is stored on ice (Huss, 1995).

The freshness quality of fish can be estimated by sensory, microbiological (TVC) or by chemical methods such as measuring volatile compounds (TVBN) and lipid hydrolysis among others (Ozyurt *et al.,* 2009). Since the consumer is the ultimate judge of the quality, microbiological and chemical method must correlate with sensory method before being used in the laboratory. Though, sensory method is the most commonly used for the quality assessment of raw fish and has always been regarded as the primary way to evaluate seafood freshness. Thus, it must be performed scientifically under carefully controlled conditions so that the effects of test environment, personal bias, etc., may be reduced. Quality index method (QIM) scheme which is an accurate and objective method of determining fish freshness, gives the reliable information of fish quality thus facilitating and enhancing management in fish processing and marketing (Martinsdóttir *et al.,* 2001). It is recognized as a reference method in sensory research because it is rapid, reliable, simple to apply, and specific for particular fish species. Also, it can be a part of labelling and identification of the catch (Hyldig and Nielsen, 2004). When applying QIM scheme,

the outer appearance of the fish (skin), gills, eyes, and texture also odour of the gills and mucus of the skin are evaluated.

Based on the evaluation approach, postmortem changes in external appearance, odour and texture are evaluated according to species related descriptors. The scheme is designed so that the quality parameters of very fresh fish receive zero points. Deterioration progress with storage time would be described with maximum of 3 demerit points at spoilage, with the quality index (QI) increasing linearly with storage time in ice. It is therefore important that fish handlers and processors minimize all handling procedures that can harm the quality of the end product (Olsen, 2004).

Microbiological method includes the enumeration of total aerobic bacteria also known as total plate count or total viable count, spoilage bacteria, and various pathogenic bacteria. Specific spoilage bacteria are gram-negative bacteria such as *Pseudomonas spp.*, *Shewanella spp*. (H_2S-producing bacteria/specific spoilage bacteria) among others. Usually the number of specific spoilage bacteria must be related to the sensory quality and the remaining shelf life (Gram *et al.,* 1990; Dergal *et al.,* 2013). Chemical method includes measurement of chemical spoilage indicators such as TVBN and lipid hydrolysis. The aim of this study was to develop and validate the Quality Index method scheme and spoilage indicators for quality management of Nile perch marketed in Tanzania.

1.2 Problem Statement and Study Justification

In Tanzania, the quality management system and safety for export of Nile perch is well established and monitored to meet international standards. Fish quality control is emphasized in export products more than in domestically consumed products. For example, implementation of Hazard Analysis Critical Control Point (HACCP) principles is only mandatory for exported fish. The quality and consumer health-related aspects in domestic markets have received little attention because no inspection and monitoring of

good hygienic fish handling and processing are applied (Kyangwa and Odongkara, 2005). Also lack of knowledge regarding improved fish handling and post-harvest practices contribute to the poor quality of fish and fishery products (FAO, 2013). Therefore, this study is intending to develop the QIM scheme for Nile perch processed for domestic markets so as to facilitate communication between buyers and sellers of Nile perch and fulfill demand of inspection authority and regulations. However, the adoption of QIM will help to identify the fresh and /or spoiled Nile perch, aid to establish the storage life and even further to predict the remaining storage life on ice so as to reduce post-harvest losses in the fisheries sector while ensuring that the fish remains with high commercial value, potentially improving food security in the sub-sector.

1.3 Objectives

1.3.1 Overall objective

To develop and validate the Quality Index method scheme and spoilage indicators for quality management of Nile perch marketed in Tanzania.

1.3.2 Specific objectives

i. To establish the QIM scheme for Nile perch processed for domestic markets.
ii. To determine the Total Viable Count (TVC) and Specific Spoilage Bacteria (SSB) of Nile perch stored on ice over time.
iii. To determine the chemical spoilage indicators (FFA, PV and TVB-N) for Nile perch stored on ice over time.

1.4 Research Questions

i. What are the Quality Index parameters for Nile perch processed for domestic market?

ii. What are the microbiological spoilage indicators of ice stored Nile perch?

iii. What are the chemical spoilage indicators for ice stored Nile perch?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Quality and Safety Assurance of Export Fish and Fish Products in Tanzania

Quality management system for fish export market is well established and monitored. At the company level, various quality assurance standards and guidelines have been translated into their food safety management systems (Kussaga *et al*., 2014, Onjong *et al*., 2014). The European Union lays down harmonized requirements governing hygiene in the capture, processing, transportation and storage of fish and fishery products, checks on finished products including organoleptic/sensory, chemical and microbiological parameters. In artisanal fisheries, the quality management system is insufficiently implemented, therefore, the possibility of contamination in fish from artisanal can even be higher because no inspection and monitoring of good hygienic fish handling and processing are applied (Kyangwa and Odongkara, 2005). This creates uncertainties to consumers regarding their safety due to the presence of poor quality fish and fish products in markets (David *et al.,* 2008). However, fish quality control standards and marketing section under Fisheries Division (FD) in the Ministry of Livestock and Fisheries is responsible for monitoring, surveillance, quality control and certifying fish and fishery products to meet national and international quality standards as enacted by the Fisheries Act No. 22 of 2003 and Fisheries Regulation of 2009.

2.2 Fish Spoilage

Fish spoilage is a change in a fish or fish product that makes it less acceptable or unacceptable to the consumer for its original intended purpose. Fish flesh provides an excellent substrate for the growth of most heterotrophic bacteria with compositional attributes that affect bacterial growth and the related biochemical activities. Fish also

possess a neutral or slightly acid pH and high moisture content, which permit the growth of a wide range of microorganisms (Huis 1996). Microbiological activity has been noted as the main cause of fish deterioration, followed by non-microbiological reaction namely: oxidative rancidity and then chemical or enzymatic denaturation of proteins. The immune system in live fish confines the bacteria on the surface of the skin and/or within the walls of the viscera. Upon death, there is a migration of bacteria into the interior of the flesh where they degrade tissue components leading to unpleasant odours and flavours associated with spoilage. When a fish is alive, its life processes are maintained by a complicated and interacting system of chemical reactions mediated by complex organic compounds and enzymes that enable the reactions to proceed smoothly and under control (Huss, 1994).

After fish dies, the rigor cause the muscle to relax, and through storage in ice, the flesh became soft due to autolysis influenced by both fish muscle enzymes and microbial enzymes (Nielsen, 1995). Also chemical reactions continue but the balance of reactions is modified for example, the onset and resolution of rigor mortis lowers and raises the pH (Connell, 1990). The change in pH affects the enzymatic and other chemical reactions in fish muscle cells, which may result in flavour and odour changes. Hydrolysis of lipids in fatty fish results in sweaty and slightly cheesy odours and flavours (Howgate, 1985). The quality deterioration of fresh fish is characterized by an initial loss of ‘fresh fish flavour’ (sweet, sea weedy). After a period where the odour and flavour is described as neutral or non-specific, the first indications of off-odours and flavours are detectable. Therefore, these will gradually become more pronounced and lead to rejection of the fish.

2.2.1 Microbiological spoilage

The muscles of fresh or live fish contains high bacterial load on the surface slime of the skin, on the gills and in the digestive tract. Bacterial loads on skin of fish from catch can

range from hundreds up to millions per square centimetre ($10^2 – 10^7/cm^2$) and in the gills and intestines in the range of $10^3 – 10^9$/g (Adams and Moss, 2008). These bacteria include Gram negatives of the genera *Pseudomonas, Shewanella, Psychrobacter, Vibrio, Flavobacterium,* and *Cytophaga* and some Gram-positives such as *coryneforms and micrococci* which are characterized by their dominance in the microflora of spoiling fish and their ability to produce spoilage compounds (Gram *et al.,* 1987). Storage of fish at lower temperature specific spoilage organisms generally grow faster than others in the natural microflora, produce the metabolites responsible for off-flavour, off-odours and finally cause sensory rejection (Huss, 1994).

The spoilage association developing in aerobically stored fish consists typically of Gram-negative psychrotrophic non-fermenting rods. Thus, under aerobic iced storage, the flora is composed almost exclusively *S. putrefaciens and Pseudomonas sp. S. putreficiens* mentioned as specific spoilage bacteria of marine temperate-water fish stored aerobically in ice and their number is inversely linearly related to remaining shelf-life of iced fish (Jorgensen *et al*., 1988). *Pseudomonas sp.* is the specific spoilers of iced stored tropical freshwater fish and is also together with *S. putreficiens,* spoilers of marine tropical fish stored in ice though *S. putreficiens* has been also isolated from tropical freshwaters but does not appear to be important in the spoilage of iced freshwater fish from tropical waters (Gram *et al*., 1990).

Most bacteria identified as specific spoilage bacteria produce one or several volatile sulphide. Example *S. putreficiens* and some *Vibrionaceae* produce H_2S from the Sulphur containing amino-acid L-cysteine (Gram *et al.,* 1987). In contrast, neither *Pseudomonas* nor *P. phospherium* produce significant amounts of H_2S. When decomposing the thiosulphate or the amino acid cysteine, these bacteria form black colonies due to precipitation of Ferrous sulfide (FeS). The number of H_2S-forming bacteria grows well at

22°C than 37°C simply because the H_2S-forming bacteria are psychrotrophic in nature. Gram *et al*. (1987) identified the majority of black colonies or H_2S-forming bacteria, as *Alteromonas putrefaciens*, which is currently known as *S. putrefaciens* and a few of them as *Vibrionaceae* due glucose fermentation. In addition, Mhongole (2009) reported that, the main spoilage organism's composition in spoiled whole Nile perch and chilled fillets include *S. putrefaciens, Vibrionaceae/ Aeromonas, Pseudomonas, and Enterobacteriaceae.*

2.2.2 Autolytic spoilage

Autolytic changes are responsible for the early quality loss in fresh fish but contribute very little to spoilage of chilled fish and fish products. Autolytic changes are contributing to spoilage mainly by making catabolites available for bacterial growth (Huss, 1995). The fish viscera contain proteolytic enzymes responsible for food digestion but when fish die, they attack the organs and the surrounding tissues which cause a condition known as belly-burst (Connell, 1990). After fish die, they become involved in predominantly degradation reactions such as hydrolysis of glycogen to lactic acid, resulting in the fall of pH from the normal neutral to acidic i.e., 7 to 5.8-6.8 depending on the species and condition of the fish. The decline in pH and the imbalance in the biochemical reactions within the fish musculature brought about by the post mortem continuation of enzymatic activity, initiates a phenomenon referred to as rigor mortis, which involves the stiffening of the fish muscles, which subsequently may pose processing problems. It affects the quality of the fish, as the texture of the flesh is rendered firmer because of its tendency to lose moisture. The increase in pH from 6.0-8.0 in some species provides a favourable environment for bacterial proliferation Therefore, the change in pH affects the enzymatic and other chemical reactions in fish muscle cells, which may result in flavour and odour changes (Connell, 1990).

2.2.3 Chemical spoilage (oxidation)

Fish have higher degree of unsaturated lipids than most other foods and therefore is susceptible to oxidative rancidity (Connell, 1990). After death, the lipids in fish are subjected to two major changes, lipolysis and auto-oxidation which constitute important chemical spoilage processes in fish (Huss, 1994). The main reactants in this process involve atmospheric oxygen and fish lipid but the reactions are initiated and accelerated by heat, light (especially UV-light). The lipid components of the post-mortem fish muscle tissues are susceptible to oxidation due to their free fatty acids, which are more unsaturated. Lipid oxidation is one of the major factors that reduce the quality and acceptability of fish and fish products because it promotes the oxidative damage of proteins through the pro-oxidant activity of primary (hydroperoxide) and secondary (aldehyde and ketones) products. The oxidation process involves the degradation of Poly Unsaturated Fatty Acid (PUFA), vitamins and other tissue components and the generation of free radicals that changes the colour and texture of meat/fish (Kanner, 1994).

2.3 Methods for Assessment of Fresh Fish Quality

There are two methods that are used to determine the quality of fish products. These are sensory and instrumental methods (Botta, 1995).

2.3.1 Sensory evaluation

It is a systematic assessment of appearance, texture, odour and flavour of food (fish). The appearance of fish such as skin, eyes and gills gives the clear idea about the freshness of the fish. It has a certain degree of subjectivity, which is only partially avoided by using an expert and trained panel (Olafsidottir *et al.,* 1997). Over the last 50 years, a large number of sensory schemes such as EU scheme have been developed for sensory analysis of raw fish. Its suitability has been questioned because using general parameters does not take

into account particular differences among species (Baixus-Nogueras *et al.,* 2003). This has led to the development of new and more specific method, (QIM) which was originally developed by the Tasmanian Food Research Unit (Bremner *et al.,* 1985).

2.3.2 Quality Index Method (QIM) scheme

QIM scheme is an accurate and objective method of determination of fish freshness. It gives the reliable information of fish quality thus facilitating and enhancing management in fish processing and marketing. QIM has been recommended for a European initiative regarding standardization and harmonization of sensory evaluation. The purpose of using QIM in research is often to find out how different ways of handling, processing and storage conditions affect the shelf life or sensory quality of the fish. QIM schemes have been developed for a number of fish species including Nile perch from Lake Victoria in Kenyan side (Okeyo, 2009). The QIM technique is based on selecting a number of significant sensory parameters or characteristic for a particular species and allocating scores to each attribute depending on the state of freshness or quality of the selected fishery products (Martinsdóttir, 2002). QIM score grades from zero being the highest score (0) which is given for very fresh fish and increasing higher scores for the fish which is deteriorating, the grading scores are (0, 1, 2 and 3). The score for all the characteristics are summarized to give an overall sensory score, so- called Quality Index It is suggested that, when the sum of score of a batch of fish reach demerit points of 10, the remaining keeping time in ice may be estimated to about five days (Huss, 1995, Martinsdóttir *et al*., 2001).

The QI increases linearly with the storage time in ice, also is an excellent tool for teaching inexperienced people to evaluate fish, training panelists and monitor performance of panelists (Martinsdóttir *et al*., 2001). Storage studies must be done to verify the results and

the sensory analysis of cooked samples used to find the end of shelf life using Quantitative Descriptive Analysis (QDA). Storage life of different fish species can be from 8 to 20 days depending on the species (Sveinsdottir *et al*., 2003). In shelf life studies, it is useful to conduct microbiological and chemical analyses in parallel to the sensory evaluation to support information about the spoilage of fish (Chytiri *et al*., 2004). If the total length of shelf life of the species on ice is known, the total number of index points can also be used to estimate the past and remaining shelf life as the QI increases linearly with the storage time on ice (Martinsdóttir *et al*., 2001). The advantage of QIM is that it is rapid and easy to perform, non-destructive to the sample and can be used as a tool in production planning and quality assurance work (Hyldig and Nielsen, 2004). The implementation of QIM scheme for all the key stages of fish value chain for evaluating the quality freshness of the fishes would help the industry to supply safe, high-quality and health fish-products (Bernardi *et al.*, 2013).

2.3.3 Microbiological methods

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 (Dergal *et al.,* 2013). The microbiology of fresh fish, ice-stored and spoiled fish from cold and temperate sea waters has been shown to be dominated by gram-negative bacteria such as *Pseudomonas spp.*, *Shewanella spp*. and *Aeromonas spp.* (Gram *et al.,* 1990). The number of specific spoilage bacteria is related to the sensory quality and the remaining shelf life of fish. However, there is no correlation between the total count and presence of any bacteria of public health significance (Gram, 1987). Masette (1999) reported that the growth of H_2S-forming

bacteria on Iron agar incubated at 37°C increased with storage time up to 10^6 cfu/g at 0˚C on day 16 and 10^4 cfu/g at 5˚C on day 19 of Nile perch storage.

2.3.4 Chemical methods

Chemical methods involve determination of the concentration of a specific chemical(s) in the food under study. Chemical methods of fish are usually used to indirectly predict the level of a sensory attribute, which allows for immediate determination of freshness (Huss, 1995). Some of the methods employed include Total Volatile Basic Nitrogen, Lipid hydrolysis and Peroxide Value.

2.3.4.1 Total volatile basic nitrogen

Total Volatile Basic Nitrogen is an important characteristic for the assessment of quality in seafood products and appears as the most common chemical indicator of marine fish spoilage. TVB-N is not reliable as an index of quality but it is a determinant of quality of fresh fish because of its close relationship with sensory score and bacterial counts (Amegovu *et al.,* 2012). It is a general term, which includes the measurement of trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile basic nitrogenous compounds associated with seafood spoilage (Huss, 1995).

Total Volatile Bases (TVB) is a group of biogenic amines formed in non-fermented food products during storage (Horsfall *et al.,* 2006). The combined total amount of ammonia (NH3), 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). The increase in the amount of TVB is parallel with the increase in TMA during spoilage. As the activity of spoilage bacteria increases after the death of fish, a subsequent increase in the reduction of trimethylamine oxide to trimethylamine is observed (Yusuf *et al.,* 2010). The source of DMA and TMA in fresh and processed fishery products is trimethylamine oxide (TMAO). Post-mortem degradation of TMAO and the subsequent accumulation of volatile amines play a key role in the quality loss of the fish products due to the objectionable odours associated with the degradation products. Dimethylamine formation is produced during frozen storage and does not have a pungent odor compared to TMA and ammonia, and is not linked to bacteria degradation (Wu and Bechtel, 2008). There are many analytical methods that have been developed for quantitative measurements of TVB-N whereby steam distillation is the most known and widely used procedures for TVB-N determination (Malle and Poumeyrol, 1989; Venugopal, 2002).

The concentration of TVB-N in freshly caught fish is typically reported to vary between 5 and 20 mg/100 g and the TVBN values increase according to time of storage (Muhammed and Sevim, 2007). In fresh Nile perch TVBN contents varied between 9 and 11 mgN/100g (Karungi *et al.,* 2004). Also, it has been proposed that the quality classification of fish and fish products regarding TVB-N values would be ‘‘high quality” up to 25 mg/100 g, ‘‘good quality” up to 30 mg/100 g, ‘‘limit of acceptability” up to 35 mg/100 g, and ‘‘spoilt” above 35 mg/100 g (Ozyurt *et al.,* 2009, Amegovu *et al.,* 2012). Also, Dalgaars (2000) suggested that the limit of TVBN from 25 to 35 mgN per 100 g of muscles have also been proposed for rejecting commercial fresh whole fish and processed fish products. TVB-N does not help to discriminate among the early stages of deterioration in fish however, in the latter stages of deterioration, they represent quite sensitive indices. Mhongole (2009) reported the TVBN level of 6-8 mgN/100 g during the first three weeks of storage in ice and increased up to 16.80 mgN/100 g after 33 days of iced whole Nile perch. Further, he

suggested that TVBN is not a reliable indicator of freshness of iced whole Nile perch as well as chilled fillets. According to Okeyo (2009), the TVBN level of 26-28 mgN/100 g flesh showed the end of acceptability of the Nile perch.

2.3.4.2 Lipid hydrolysis

Lipid hydrolysis is another chemical reaction that affects the quality of fish during storage. Fat hydrolysis breaks down the acyl groups of triglycerides and produces free fatty acid (Adawiyah *et al.,* 2012). Lipid hydrolysis taking place during cold storage is an important change that occurs in fish muscle lipid post-mortem with accumulation of FFAs. Lipid hydrolysis progress depends on different factors, such as the amount of lipid present, the degree of unsaturated fatty acids in the muscle, salt composition and storage conditions of products (Nguyen *et al.,* 2012). FFAs content is considered as a crucial factor and is linked with the quality as well as the economic value of the edible oil. However, there is a gradual increase in the FFAs content with increasing storage time and it could act as a good indicator for the assessment of the freshness of the Nile perch (Mahesar *et al*., 2014). The accumulation of FFAs could be attributed to lipases and phospholipases activity in Nile perch muscle, digestive organs as well as microorganisms which enhanced with extended storage. However there is a correlation between the FFAs and the TVC and H_2S producing bacteria. Okeyo (2009) reported that, the FFAs levels for Nile perch can reach up to 2% on day 22 of ice storage, where by at this point fish perceived by the sensory panel to be unacceptable for human consumption which makes the FFAs to have high correlation with the QI. Lipid hydrolysis activity is determined by measuring the free fatty acid (FFAs) level in the total extracted lipid (Bligh *et al.,* 1988).

2.3.4.3 Peroxide value (PV)

Peroxide value measures the concentration of peroxides and hydroperoxides formed in the initial stages of the lipid oxidation and it is widely used for the estimation of oxidative

rancidity in fats and oils (Olafsdittir *et al*., 1997). The total of hydroperoxide content is one of the most common quality indicators of fats and oils during production and storage (Nguyen *et al.,* 2012). Detection of peroxide gives the initial evidence of rancidity in unsaturated fats and oil. It is determined by measuring the amount of iodine which is formed by the reaction of peroxide (formed in fats and oil) with iodide ion. It results from the chemical deterioration of fats whereas other deteriorative reactions such as microbial or enzymes attacks can largely controlled by lowering the temperature, but not helpful in preventing oxidation since low threshold are involved (Azhar and Nasa, 2006). The formation of hydroperoxides as primary lipid oxidation products may break down to a variety of non-volatile and volatile secondary products that are determined by peroxide value measurement (Shahidi and Zhong, 2005). However, a number of methods have been developed for determination of PV, among which the iodometric titration, ferric ion complex measurement spectrophotometry, and infrared spectroscopy are most frequently used (Shahidi and Wanasundara, 1998).

2.4 Shelf Life of Fresh Fish Stored on Ice

Shelf life is established by the duration between the time of capture and the time when the cooked quality score dropped below the limit of acceptability. Also, it can be defined as number of days that whole, fresh (gutted) fish can be stored in ice until it becomes unfit for human consumption. The shelf life of fresh fish products is influenced by a number of factors, such as the initial microbial load, the fishing method and the post-harvest handling of the catch, storage conditions, fat/water content and varies from species to species (Huss, 1995). The shelf life of freshly caught whole Nile Perch reported to be about 28-33 days on ice (Mhongole, 2009).

World market demand for a supply of safe and healthy food is increasing that is why food preservation is important to increase shelf life and to maintain nutritional value and

quality. Therefore, the ability to predict shelf life of fish products is of interest (Kaale *et al.*, 2011). Shelf life of fish and fishery products is a key factor in the fish industry because it allows processors to plan how to process and transport products to different markets. Handling practices, processing contamination and storage conditions affect the shelf life of fish and fishery products Also temperature fluctuation is a key factor that greatly affects quality and shelf life of fish products during processing, transportation and storage in retail shops (Huss, 1995). Gutting can extend the shelf life in ice stored fish by the average of 4 days. H_2S producing bacteria for ungutted fish rich 6.0 log cfu/g in 24 days in ice while the same level reached on day 28 in the gutted fish (Lokuruka, 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

This study was conducted in Mwanza region specifically in Ilemela and Nyamagana Districts. Fish samples were collected from the fishermen (Igombe landing site), fish factories (Victoria perch Ltd and Tanzania Fish Processors Ltd) and cold stores (Nata, Kirumba and Mkuyuni). The Igombe landing site was selected because it was easily accessible and has relatively high availability of Nile perch. The Victoria Perch Ltd and Tanzania Fish Processers Ltd were not specifically selected but are where I managed to get the reject fish. Other sampling sites such as Nata, Kirumba and Mkuyuni were selected because they are among the main supplier of frozen Nile perch to the domestic and regional markets. Analysis was done at an accredited National Fish Quality Control Laboratory (NFQCL) –Nyegezi, Mwanza.

3.2 Study Design

A cross sectional and experimental study designs were used in this study. The study involved sample collection, experimental set up and laboratory analysis of different parameters identified under this study.

3.3 Sampling and Storage of Fish

A total of 118 fish samples were purchased from the fisherman at landing site, fish factories and from cold stores. In this study 68 fish were from the fisherman at the landing sites, 20 from the fish factories and 30 from cold stores. The number of sample collected from the landing site was high compared to other sampling stations because samples from the landing site were used to develop the QIM and for shelf life study. Only fish that were

not qualified to be processed for exportation were collected from the fish factories. The weight and length of each fish sampled were recorded. The average weight and length of samples were 1800 grams (g) and 52 centimetre (cm), respectively. All the collected samples were iced immediately and stored in cool boxes, then transferred to the NFQCL for analysis. Fish samples from the landing site were divided into two batches with one batch gutted and the second batch ungutted. The gutted fish was washed with the running tap water to remove the residual blood. Both gutted and ungutted fish were separately packed in polyethylene bags, labeled and then placed in Styrofoam boxes containing flake ice.

The boxes were placed in refrigerator at 0 to -1˚C to maintain the temperature during the entire period of the study on the quality changes during storage so as to develop the QIM scheme and also for shelf life study. The ice and fish ratio (1:1) was maintained constant throughout the experiment and any melted ice was replaced. Fish samples collected from the fish factories and from cold stores were iced then transported to the laboratory for analysis on the same day of sampling. All samples were analyzed for sensory, TVC, SSB, TVB-N, FFAs and hydroperoxide value.

3.4 Laboratory Analyses

3.4.1 Sensory analysis of raw and cooked Nile perch fish

3.4.1.1 Establishment of the QIM scheme for Nile perch processed for domestic market

The QIM scheme for Nile perch processed for domestic market were developed using fish samples collected from the fishermen at the landing site, following the 3 steps of QIM scheme development according to the methodology earlier described by Martinsdottir *et al.* (2001), Hyldig *et al.* (2007).

The first step included the preparation of the preliminary QIM scheme for sensory evaluation of fresh Nile perch. Twelve experts (4 men and 8 women) were selected among the staff of the NFQCL and Fisheries Education and Training Agency (FETA), Nyegezi, experienced in sensory evaluation and QIM for fish. The panelists required to evaluate two batches of Nile perch to design the QIM. Samples from each batch were presented to the panel in random order and each member was required to evaluate eight (8) samples in each batch from week 1 to week 4 stored on ice. Major sensory indicators including appearance, gills, eyes, odour and texture were selected among the listed attributes in preliminary scheme. All observations were carried out following the general guidance for the design of test room and testing conditions described in ISO 8589 (2007). A score from 0 to 3 demerit points was given for every change of evaluated parameter. All suggestions of improvements by the panel members in the evaluation were included in the final scheme.

The second step of the QIM development included the training of the panelists and testing of the preliminary scheme. A panel consisted of 10 assessors (3men and 7 women) from NFQCL and FETA. The selection and training of the panel members were carried out in 3 training sessions according to ISO 8586 (1993). The preliminary scheme was explained to the panel members during the first training session and the panelists were familiarized with two groups of Nile perch in different stages of freshness stored on ice. The testing of the preliminary scheme was done at fixed time intervals, from week 1 to week 4 on ice and every panel member was required evaluating eight (8) samples from each batch. Panel leader prepared the final version of the QIM scheme by taking into account all comments, suggestions, and improvements made by the panel members.

The third step included the validation of the QIM scheme through shelf life study of the samples from landing sites. Six fish (3 whole and 3 gutted) were sampled in each sampling

day (1, 7, 14, 21, 26, 29 and 33). Sampling was done for the interval of 7 days from day 1-21 and one the spoilage detected sampling days were reduced so as to monitor the shelf life. Fish samples with unknown storage history was placed on the evaluating table, coded with 3 digit random numbers and presented to the panelist for evaluation. All the panelists were required to assess every sample according to the newly developed QIM scheme. The data obtained in this step were used to calculate the calibration curve of QI scores. During the shelf life study, sensory, chemical and microbiological indices were measured to follow the spoilage pattern.

3.4.1.2 Sensory evaluation of the cooked Nile perch

This was done to determine the perception of the panelists on different organoleptic properties of the cooked fish such as odour, taste and texture. The fish flesh (200g) from each fish in each sampling day was cut, washed then chopped into small pieces of 20g each and wrapped in aluminum foil individually and then cooked at 95°C for 5 minutes (min). After cooking, the samples were left to cool and immediately presented to the panelists with previous experience in fish sensory assessment. The sensory assessment score sheet for cooked fish was adopted from Huss (1995) structured acceptability scale of 0-10 where by an average score ≤ 4 was used as the sensory rejection point, for both groups of fish (Appendix 1).

3.4.2 Microbiological analysis

Determination of total viable count and specific spoilage bacteria

(i) Sample preparation

During sample preparation, skin was removed aseptically and 25 g of the flesh was chopped using sterile scalpels and forceps and mixed with 225 ml of 0.1% Buffered

Peptone Water (BPW) (HiMedia Laboratories Pvt. Ltd Mumbai, India) in a sterile stomacher bag. The mixture was homogenized using stomacher for 120 seconds to obtain 1:10 dilution and was serially diluted as required. Tenfold serial dilution was made using 0.1% BPW in prepared sterile test tubes. Sterile duplicate petri dishes were labeled according to the dilution index.

(ii) Enumeration of TVC and SSB

The TVCs was enumerated using pour plate method in Iron agar as described by Gram *et al*. (1987). One milliliter (ml) from serial dilutions was pour plated in duplicates with approximately 10ml Iron agar (Oxoid, Hampshire, England) tempered at 45˚C and mixed thoroughly by rotating the petri dishes and the mixture was allowed to solidify and again approximate 15ml of Iron agar was added as an overlay and allowed to solidify. After complete solidification, prepared dishes were inverted and placed in the incubator at 22˚C for 72 hours (h). After incubation time, both typical black and white colonies were counted using colony counter and computed as per ISO 4833:2003 and reported as colony forming unit per gram (cfu/g).

The counted and number of cfu/g was calculated using the following formula

$$\frac{CFU}{g} = \sum C / V\,(n_1+0.1(n_2)\,d \qquad (1)$$

Where; ∑C is the sum of colonies counted on the dishes retained

n_1 is the number of dishes retained in the first dilution

n_2 is the number of dishes retained in the second dilution

d is the dilution factor corresponding to 1st dilution

v is the volume of sample inoculated.

The black colonies that represented the bacterial population producing H_2S were calculated and reported separately as SSB in cfu/g. The sum of black and white colonies was reported as TVC in cfu/g. The cfu/g was further converted into $\log_{10}$ cfu/g.

(iii) Identification and biochemical tests

The typical black colonies were streaked on Nutrient agar (HiMedia Laboratories Pvt. Ltd Mumbai, India) and incubated at 37˚C for 24 h. After incubation, purified colonies were tested for oxidase and catalase. Catalase test was done using 3% H_2O_2 and production of oxidase using oxidase reagent (Kovacs, 1956). The isolates were preserved in 50% glycerol and stored for further confirmations.

(a) Catalase test

The small colony of the organisms from the petri dish containing the overnight culture was taken using inoculating tube then placed on the slide. Two drops of 3% hydrogen peroxide (H_2O_2) solution was added on the test smear then the bubble formation was observed using the hand lens. Colonies that showed evidence of gas formation were designated as catalase positive while the one showed no evidence of gas formation were designated as catalase negative.

(b) Oxidase test

A loopful of one day old culture was spread on the oxidase discs. The reaction was observed within 5-10 seconds (s). A change later than 10s or no change at all was considered as negative reaction. In a positive reaction the enzyme cytochrome oxidase combines with N, N-dimethyl-p-phenylenediamine oxalate and a-naphthol to form the dye, indophenol blue.

3.4.3 Chemical analysis

3.4.3.1 Determination of total volatile basic nitrogen

TVB-N was determined by using steam distillation method as described by Malle, and Poumeyrol, (1989). Sample (100g) of skinless muscle from the dorsal region of each fish was cut using knife then weighted in duplicate and placed into a laboratory blender followed by 200 ml of 7.5% Trichloroacetic Acid (TCA) and blended. Thereafter, 75ml of filtrate was filtered using whatman filter paper no.1 and then 25 ml of filtrate was placed into a distillation flask followed by addition of 6 ml of 10% alkaline Sodium Hydroxide (NaOH). The mixture was distilled then collected into conical flask containing 10 ml of 4% Boric (with 0.04 ml mixture of methyl red and bromocresol green indicator). The distillation was continued until the final volume of 100ml of the mixture was collected. The volatile bases were titrated against 0.025N Sulphuric acid (H_2SO_4) until the green colour changed to pink. The amount of Sulphuric acid used was measured as total bases distilled over. The TVB-N was calculated and expressed as mg nitrogen/100g of fish muscle using the following equation;

$$\text{TVBN mgN}/100\text{g} = (\text{V} \times \text{C} \times 14 \times 300)\ /25 \quad \text{.....(2)}$$

Where V is the volume of acid added

C is the concentration of sulphuric acid used

25 represent ml of filtrate

14 is the molecular weight of nitrogen.

3.4.3.2 Extraction of lipid

The PV and FFAs content were determined in the extracted lipid. PV and FFAs were determined according to the method of Egan *et al.* (1981) and expressed in milliequivalent (mEq) peroxide per kg of lipid and percentage of oleic acid, respectively. Sample (30g) of skinless fish muscle was weighed in duplicate, blended using laboratory blender then

placed in 250 ml of Scott bottle. Afterwards, 100 ml of chloroform was added and left for overnight to extract the lipid. The following day, the aliquot chloroform was filtered using whatman filter paper no. 40.

(i) Determination of free fatty acids

This was done to monitor the extent of hydrolysis of fish lipids during ice storage. FFAs content was determined by the method described by Bernardez *et al*. (2005). 15 ml of aliquot chloroform extract was pipetted into Erlenmeyer flask followed by addition of 50ml of 95% Ethanol while shaking to dissolve the sample. Thereafter, 3 drops of phenolphthalein indicator solution were added followed by titration with 0.05M Sodium hydroxide solution while shaking vigorously until the permanent pink colour appeared and persisted for 1min. The FFAs content was expressed as percentage oleic acid. Therefore, the percentage of FFAs as oleic acid was calculated as follows;-

$$\text{Percentage of FFA as oleic acid} = (\text{V} \times \text{N} \times \text{M})/10\text{x w} \quad \ldots\ldots(3)$$

Where V is Volume (ml) of NaOH used

N is Normality of NaOH used

M is Molecular weight of FFAs (oleic acid=282/10 or 28.2)

w is weight of sample in g

(ii). Determination lipid hydroperoxide (PV)

The volume of 20 ml aliquot of chloroform was pipetted into an Erlenmeyer flask followed by addition of 30 ml of acetic acid. The mixture was shaken vigorously, and then 0.5 ml of saturated potassium iodide solution was added and kept in the dark for 5 min. Thereafter, 30 ml of distilled water was added and the mixture was shaken again to liberate the iodine from chloroform layer. The liberated iodine was titrated with standardized 0.01N sodium thiosulfate ($Na_2S_2O_3$) solution by vigorously shaking until the yellow color almost disappeared. Then 1 ml of starch solution (1%, w/v) was added as an

indicator then the titration was continued with 0.01N Sodium thiosulphate by vigorous shaking to release all iodine (I_2) from chloroform (CH_3CI) layer until the blue colour disappeared. The PV was calculated using the following formula;

$$PV = S \times M \times 1000 \, / g \text{ of sample.} \quad \text{.....(4)}$$

Where S is ml of Sodium thiosulphate (blank corrected)

M is Molarity of Sodium thiosulfate

g is a weight of sample

3.5 Statistical Analysis

All data were entered and stored in Microsoft Office Excel. The data were analyzed using Excel. The means for sensory parameters were analyzed using descriptive statistics while one way Analysis of variance (ANOVA) was used to compute the means of the microbiological and chemical parameters. The significant difference was judged at $p<0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Sensory Analysis

4.1.1 The QIM scheme

The QIM scheme was developed for whole ungutted and gutted Nile perch. The developed QIM scheme for ungutted Nile perch had 4 parameters and 11 sensory attributes with a total maximum score of 24 demerit points. The QIM scheme for gutted Nile perch had 4 parameters and 10 sensory attributes with a total maximum score of 23 demerit points after removing the belly flap, which was considered inappropriate for gutted fish. The score for all the characteristics are summarized to give an overall Quality Index (QI) or demerit point score. The parameters for QIM schemes for ungutted and gutted Nile perch were suggested based on the knowledge of the panel of assessors comprised of fish inspectors, fish technologists and fisheries officers from NFQCL and FETA (Table 1 and 2).

Table 1: The QIM scheme for ungutted Nile perch stored on ice over 33 days

Parameters	Sensory attributes	Quality attributes	Demerit points
Appearance	Skin	Silvery/very bright/bright/shining	0
		Slightly bright	1
		Dull	2
	Slime/mucus	Clear/transparent	0
		Slightly opaque	1
		Milky /opaque	2
	Smell	Fresh, seaweed /fishy	0
		Neutral	1
		Slightly off-odour	2
		Off-odour/rancid	3
	Scales	Intact	0
		Slightly intact	1
		Loose	2
	Belly flap	Flat/ normal	0
		Protrude/swollen	1
Eye	Clarity	Bright pupil, clear/transparent cornea	0
		Slightly opaque	1
		Opaque	2
	Shape	Convex	0
		Flat	1
		Concave/sunken	2
Texture	Elasticity/ Stiffness	Elastic /Firm/ Stiff	0
		Soft /oadema	1
		Very soft	2
Gills	Colour	Bright red/reddish	0
		Pale red/slightly discoloured	1
		Discoloured/ bleached	2
		Brownish	3
	Odour/Smell	Fresh, sea weedy/ fishy	0
		Neutral	1
		Off-odour	2
	Slime	Clear /transparent	0
		Slightly opaque	1
		Cloudy	2
		Brown	3
		Total demerit points	**24**

Table 2: The QIM scheme for gutted Nile perch stored on ice over 33 days

Parameters	Sensory attributes	Quality attributes	Demerit points
Appearance	Skin	Silvery/very bright/bright/shining	0
		Slightly bright	1
		Dull	2
	Slime/mucus	Clear/transparent	0
		Slightly opaque	1
		Milky /opaque	2
	Smell	Fresh, seaweed /fishy	0
		Neutral	1
		Slightly off-odour	2
		Off-odour/rancid	3
	Scales	Intact	0
		Slightly intact	1
		Loose	2
Eye	Clarity	Bright pupil, clear/transparent cornea	0
		Slightly opaque	1
		Opaque	2
	Shape	Convex	0
		Flat	1
		Concave/sunken	2
Texture	Elasticity/ Stiffness	Elastic /firm/ stiff	0
		Soft /oadema	1
		Very soft	2
Gills	Colour	Bright red/reddish	0
		Pale red/slightly discoloured	1
		Discoloured/ bleached	2
		Brownish	3
	Odour/Smell	Fresh, seaweed / fishy	0
		Neutral	1
		Off-odour	2
	Slime	Clear /transparent	0
		Slightly opaque	1
		Cloudy	2
		Brown	3
		Total demerit points	**23**

4.1.2 Shelf life of iced gutted and ungutted Nile perch

Shelf life study was conducted for both gutted and whole Nile perch from day 1 to day 33 on ice. The sensory evaluation was carried out according to the freshness ratings using the developed quality assessment scheme. The demerit score was given from 0-3, zero (0)

being the highest score and was given for very fresh fish and increasing scores (2-3) for the spoiled fish. The increase in the QI scores with storage days on ice was observed for both gutted and ungutted Nile perch. The freshness quality was observed to deteriorate as per storage time for all quality attributes. The major QI score contributors during the shelf life study were observed as change of the general appearance of the fish gills, eye clarity, skin and texture which showed linear relationship whereas parameters such as eye shape, slime and belly flap fluctuated with storage time.

4.1.2.1 Changes on the appearance of the gills colour

The changes of the gills colour were similar for both gutted and ungutted Nile perch from day 1-21 of storage while for ungutted Nile perch increased from 2.0-3.0 from day 26-33 while the gutted increased from 2.0-2.3 for day 29-33 (Fig. 1).

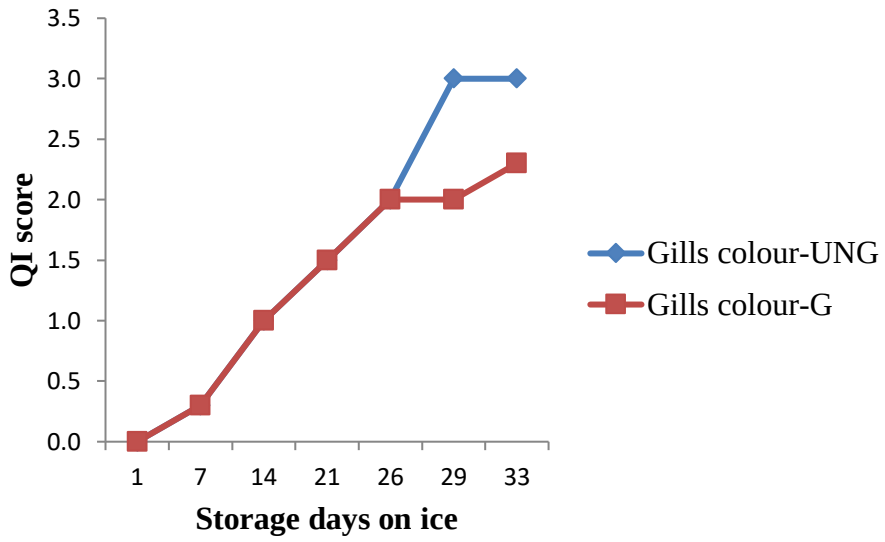

Figure 1: Quality changes in gills colour of Nile perch stored on ice over a period of 33 days estimated by sensory analysis

The appearance of gills colour was bright red from day 1-7, with QI scores of 0.0-0.3, which was described as a very fresh fish, then the colour changed to pale red from day 14-

21 with QI score of 1.0-1.5 and finally to brownish colour on day 29-33 with QI scores of 2.0-2.3 for gutted and 3.0 for ungutted. At this point the fish was judged as spoiled (Fig. 2 and Fig. 3).

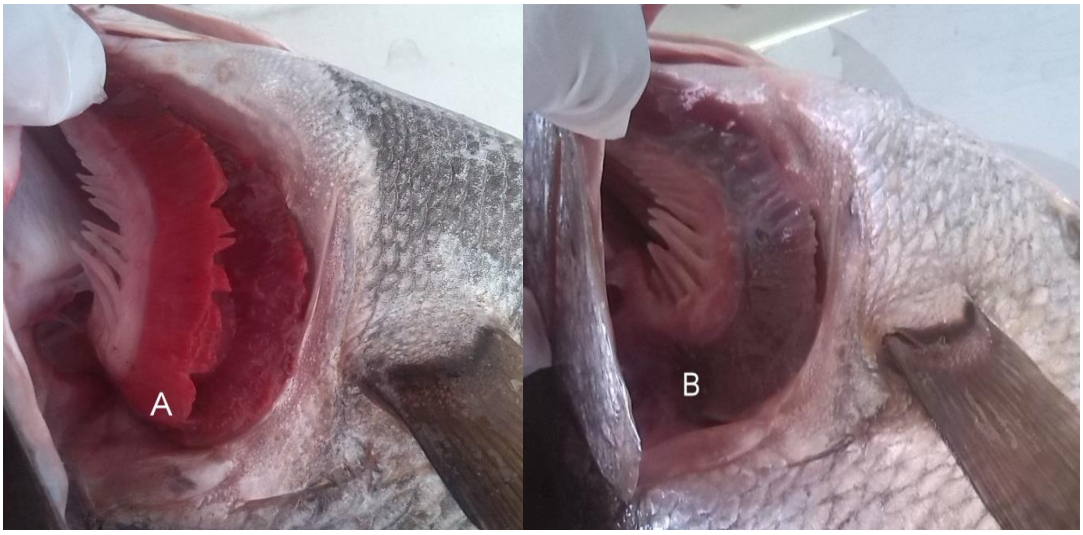

Figure 2: The appearance of the gills colour for ungutted Nile perch stored on ice. Letter "A" shows the bright red colour on day 1 and letter "B" shows the brownish colour as observed on day 33 of storage

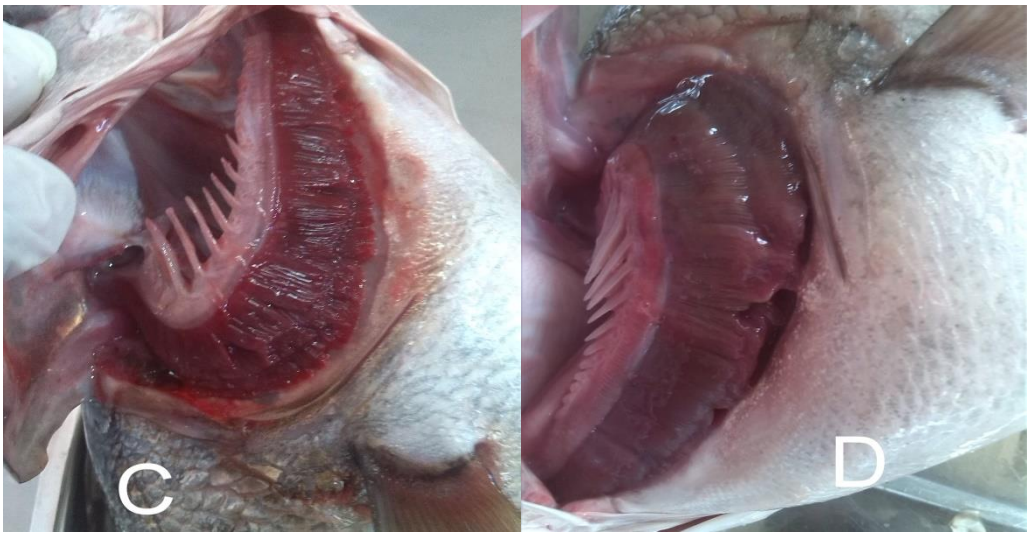

Figure 3: The appearance of the gills colour for gutted Nile perch stored on ice. Letter "C" shows the bright red colour on day 1 and letter "D" shows the brownish colour as observed on day 33 of storage

4.1.2.2 Quality changes on the appearance of the gills odour

The gills odour was changed from fresh/seaweed/fishy for very fresh fish and off-odour for spoiled fish. The changes in smell increased rapidly for ungutted compared to gutted Nile perch. For ungutted Nile perch, the fish fresh odour was from day 1-7 with QI score of 0.0-0.7, and then increased drastically up to 2.0 at the end of storage time (33 days). For the gutted Nile perch, the fish fresh odour was from day 1-14 with QI score 0.0-0.7 and then increased drastically up to 2.0 at the end of storage time of storage (Fig. 4).

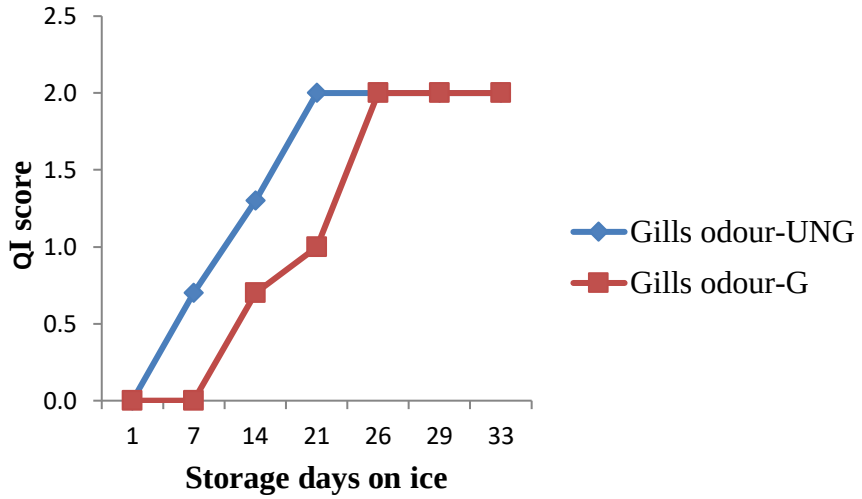

Figure 4: Quality changes in gills odour of Nile perch stored on ice over a period of 33 days as estimated by sensory analysis

4.1.2.3 Quality changes in texture

The texture test was carried out by pressing the finger on the fish to evaluate the stiffness/elasticity. When the pressed fish recovered quickly, the flesh was described to be elastic/firm and when recovered slowly meant the flesh was soft/oadema and when it did not recovered it meant the flesh was very soft. The texture of the fish flesh was changed with storage time from stiff/elastic for very fresh fish to very soft for spoiled Nile perch. The maximum QI for this parameter was 2.0. For the gutted Nile perch, the texture was good from day 1- 14 with QI score 0.0, then increased up to 1.00 from day 14 and

maintained up to day 33 of storage. For the ungutted Nile perch, the texture changed rapidly from elastic to soft and finally to very soft with QI score of 2.0 on day 33 of storage. The cut off point for this parameter was QI of 1.0 (Fig. 5).

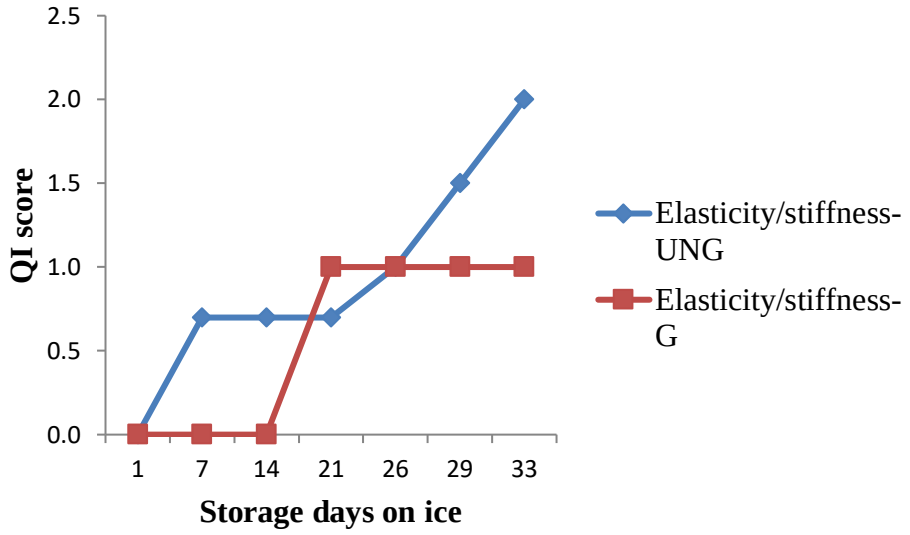

Figure 5: Quality changes in texture of Nile perch stored on ice over a period of 33 days estimated by sensory analysis

4.1.2.4 Quality changes on the appearance of eye clarity

The appearance of eye clarity (pupil and cornea) for ungutted and gutted fish changed from clear/transparent for a very fresh fish and slowly to opaque for spoiled fish (Fig. 6 & 7).

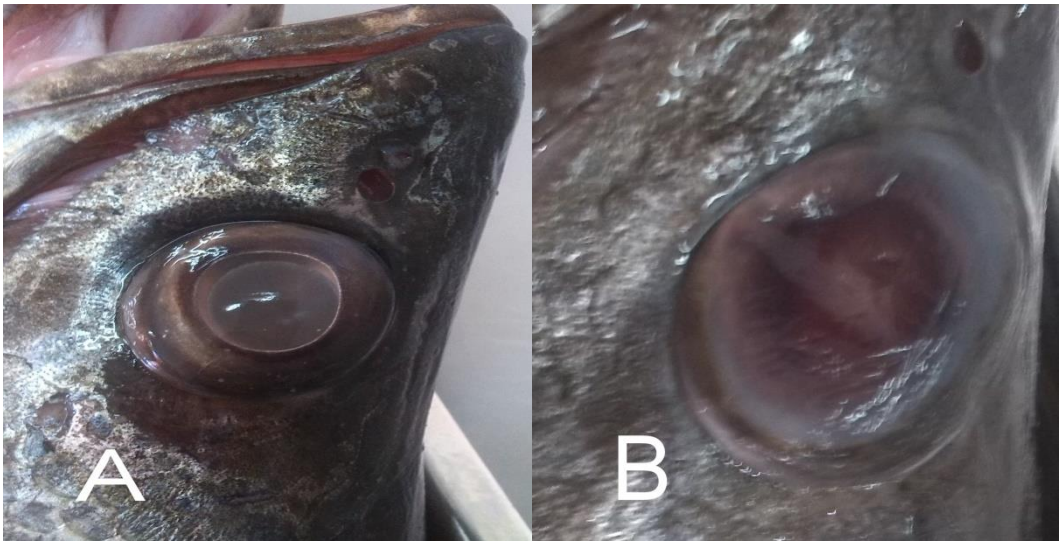

Figure 6: Quality changes in appearance of eye clarity for ungutted Nile perch stored on ice over a period of 33 days estimated by sensory analysis. The letter “A” shows the eye was convex with bright pupil and transparent cornea on day 1 while the letter “B” the eye was flat with opaque pupil and cornea on day 33 of storage

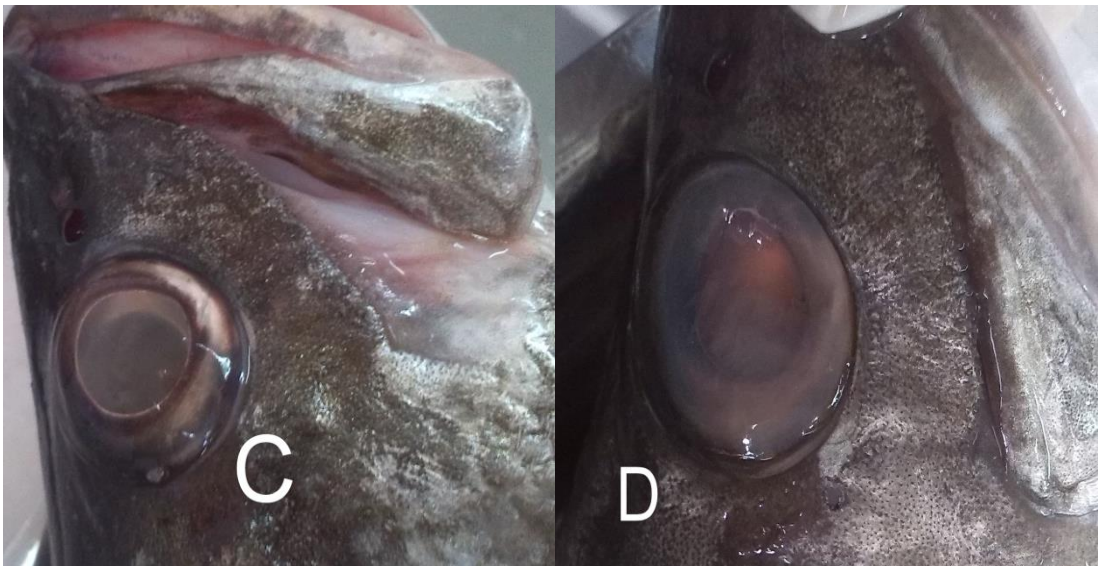

Figure 7: Quality changes in appearance of eye clarity gutted Nile perch stored on ice over a period of 33 days estimated by sensory analysis. The letter “C” shows the eye was convex with bright pupil and transparent cornea on day 1 while the letter “D” the eye was flat with opaque pupil and cornea on day 33 of storage

The maximum QI score for this parameter was 2.0. The pupil and cornea for gutted Nile perch changed slowly from day 1-7 with QI score of 0.0-0.3 then maintained to1.0 from day 14-26 and finally increased up to 2.0 in day 29-33 storage time. For the ungutted Nile perch, the pupil and cornea changed rapidly from day 1-7 with QI score of 0.0-1.0 then maintained to 1.0 from day 7-26 and finally increased up to 2.0 on day 33 of storage. Generally, the difference in score for gutted and ungutted fish was observed only on day 1-7 but was the same from day 14-33 of storage (Fig. 8).

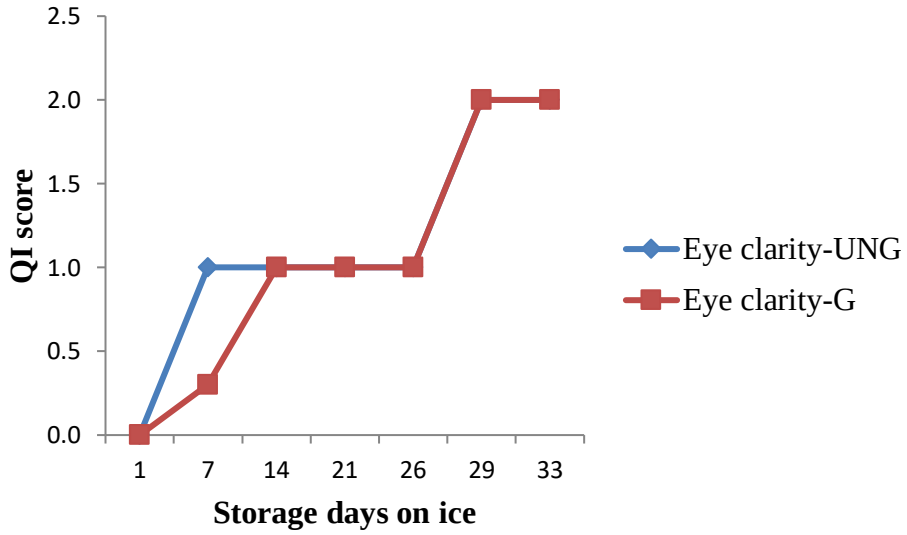

Figure 8: Quality changes in eye clarity of Nile perch stored on ice over a period of 33 days estimated by sensory analysis

4.1.2.5 Quality changes in the appearance of the skin colour

The appearance of the skin colour changed from bright shining/silvery to dull on day 33 of storage (Fig. 9A & B).

Figure 9: Changes in the appearance of the skin colour during storage of Nile perch over a period of 33 days on ice. The photo that labeled by letter "A" represent a fresh Nile perch with bright shining/silvery skin in day 1of storage while the photo labeled by letter "B" represent a spoiled Nile perch with dull skin in day 33 of storage

The maximum QI score for skin colour was 2.0. For the gutted Nile perch, the skin was bright from day 1-14 with QI of 0.0-0.3 and changed to slightly bright from day 21-26 with QI of 1.0 then changed to dull on day 29-33 of storage with the QI of 2.0. The skin colour for ungutted fish was bright from day 1-7 with QI of 0.0-0.3 and changed to slightly bright from day 14-21 with QI of 1.0, then finally to QI of 2.0 for day 29-33 of storage. The skin colour for gutted fish changed slowly compared to ungutted Nile perch although both scored 2.0 at the end of storage time (Fig. 10). Comparison between quality index and storage days for Nile perch kept on ice showed a linear relationship (Fig. 11).

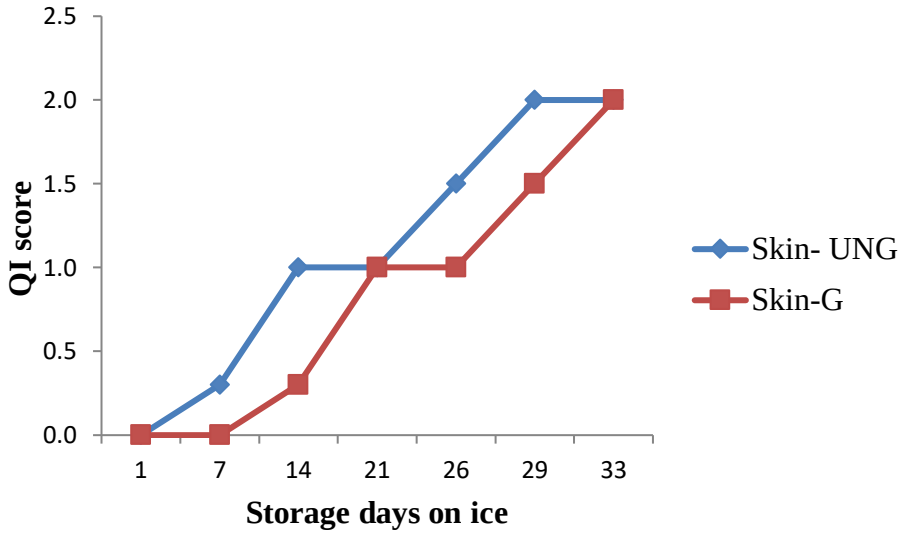

Figure 10: Quality changes in skin appearance of Nile perch stored on ice over a period of 33 days estimated by sensory analysis

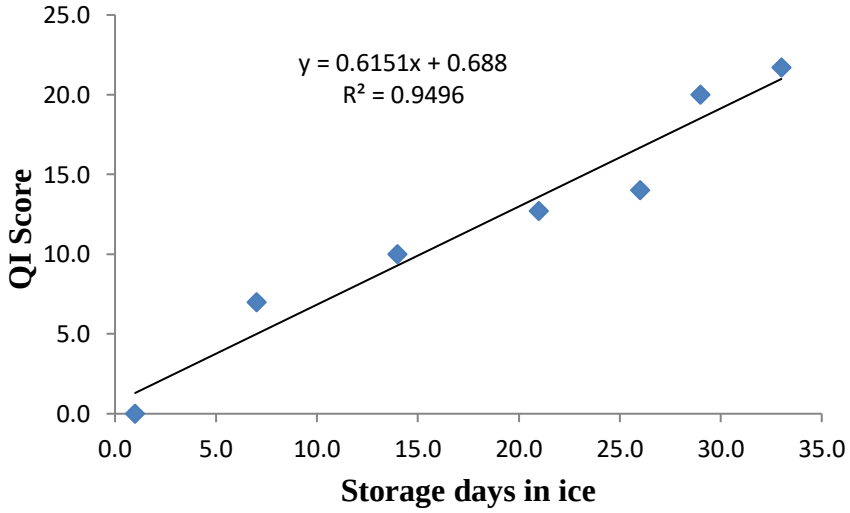

Figure 11: Comparison between quality index and storage days for Nile perch kept on ice

4.1.3 Sensory evaluation of cooked Nile perch

The structured acceptability scale for organoleptic properties of cooked gutted and ungutted Nile perch was used. A score of ≤ 4.0 for the organoleptic attributes was considered the rejection threshold level for the fish (Fig. 12).

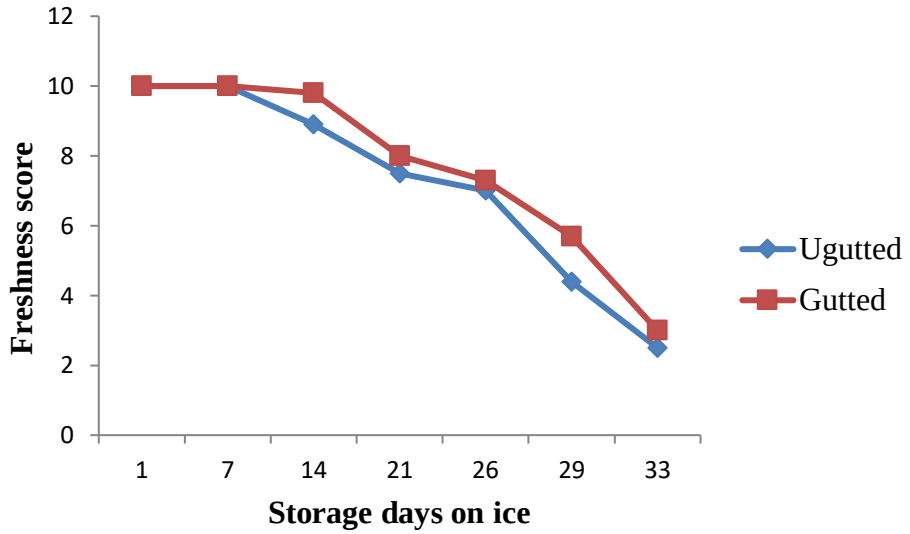

Figure 12: Changes in freshness score of cooked Nile perch stored on ice over 33 days

The sensory score of the cooked Nile perch decreased linearly with storage time. The fresh flavour and odour characteristic of the fish was strong for 1–14 days and slowly decreased until the fish became unacceptable in day 26-33. At this stage, the fish odour was stale and sour, while the flavour was changed to off- flavour and the texture was very soft and slippery.

4.2 Microbiological Analysis

The Total Viable Counts was higher than to Specific Spoilage Bacteria counts for the first days and at the end of storage time (33 days). The SSB started with relatively low count but rapidly doubled (2.16 - 4.53 $\log_{10}$ cfu/g) compared to TVC (3.33 -5.16 $\log_{10}$ cfu/g) within the first two weeks of storage on ice. In day 14, the TVC and SSB count were 5.16 and 4.53 $\log_{10}$ cfu/g, respectively where the QI score was 10. The TVC count for gutted and ungutted fish at the end of storage time (33 days) were 6.93 and 7.60 $\log_{10}$ cfu/g, respectively. The SSB count for gutted and ungutted fish increased up to 6.83 and 7.36 $\log_{10}$ cfu/g with the fish judged spoiled, when the QI score were 18 and 21.7, respectively (Fig. 13 and 14).

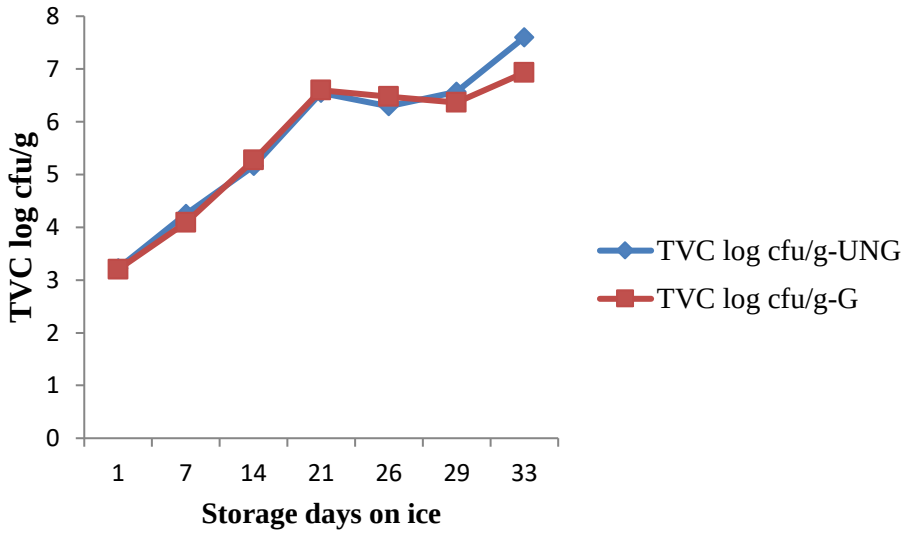

Figure 13: Total Viable Counts over the storage days for both gutted and Nile perch

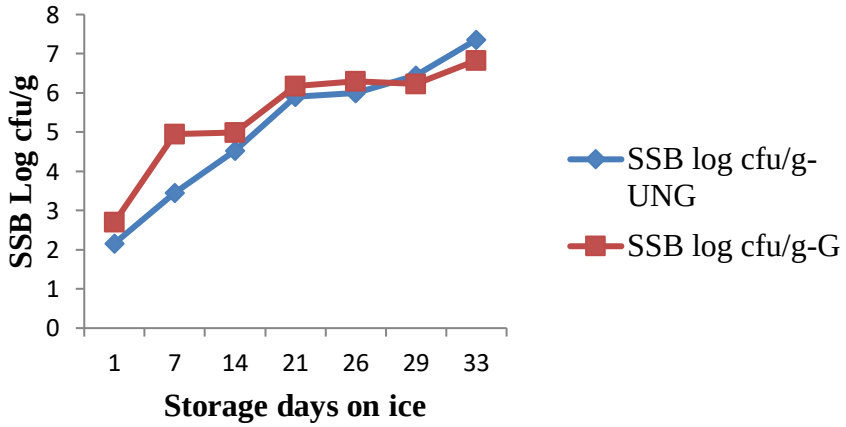

Figure 14: Specific spoilage bacteria counts over the storage days for gutted and ungutted Nile perch

The TVC) and SSB counts for both gutted and ungutted Nile perch increased linearly with the storage time (Fig. 15 and 16).

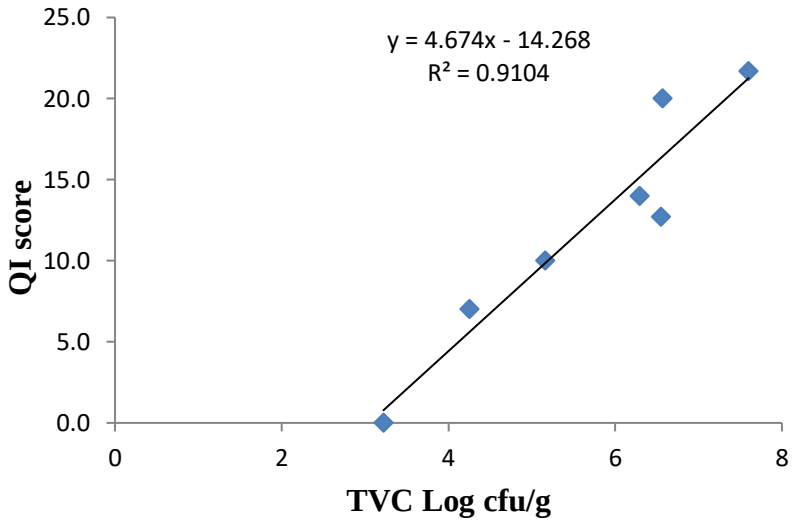

Figure 15: Relationship between Total Viable Count and Quality Index scores of Nile perch stored on ice

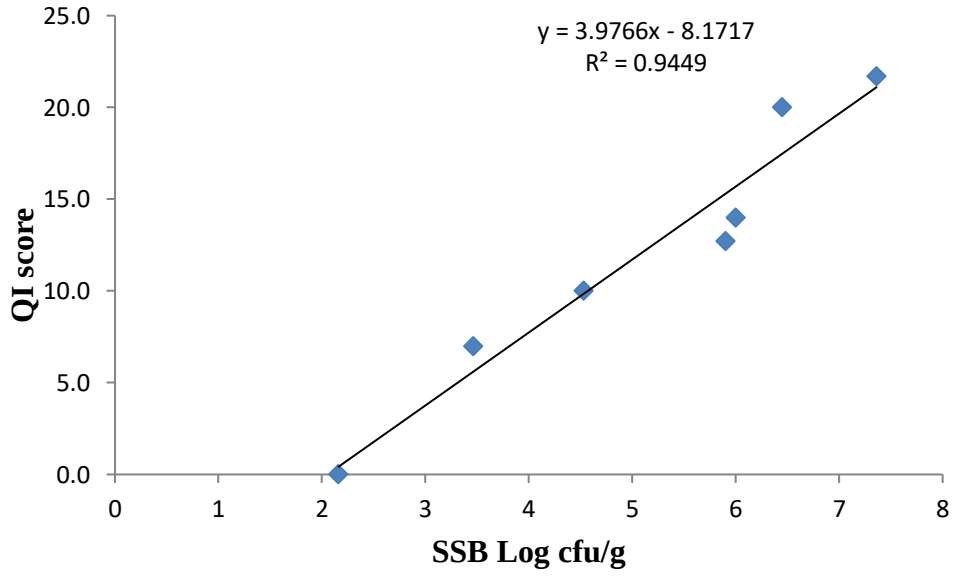

Figure 16: Relationship between Specific Spoilage Bacteria and Quality Index scores of Nile perch stored on ice over a period of 33 days

4.3 Chemical Analysis

4.3.1 Total volatile basic nitrogen

The Total Volatile Basic Nitrogen level for both gutted and ungutted Nile perch increased with QI score as well as storage days although increased slowly. The level of TVBN for gutted and ungutted Nile perch was 5.04 mg/100 g and 7.14 mgN/100 g of flesh for day 1 then increased up to 11.48 mgN/100 g and 12.18 mgN/100 g of flesh on day 33 of storage, respectively (Fig. 17).

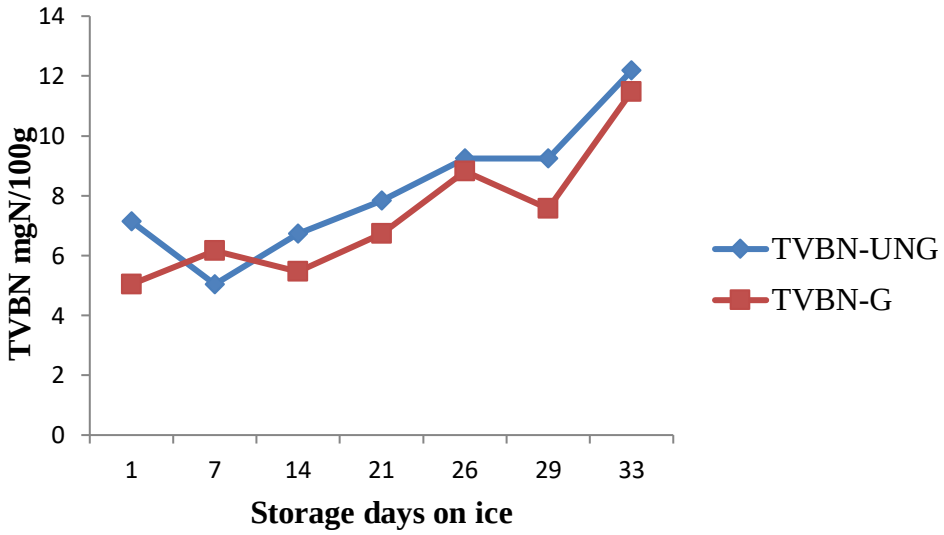

Figure 17: Total volatile basic Nitrogen and storage days for gutted and ungutted Nile perch

4.3.2 Free fatty acids

The Free Fatty Acids for both gutted and ungutted was constant (0.01-0.02%) from day 1-33 of storage. Therefore storage on ice for 33 days had no effect on FFAs (Fig 18).

4.3.3 Peroxide value

The Peroxide Value increased gradually for ungutted Nile perch up to 21 days whereas, rapid increase was observed in gutted Nile perch up to 14 days (Fig. 18).

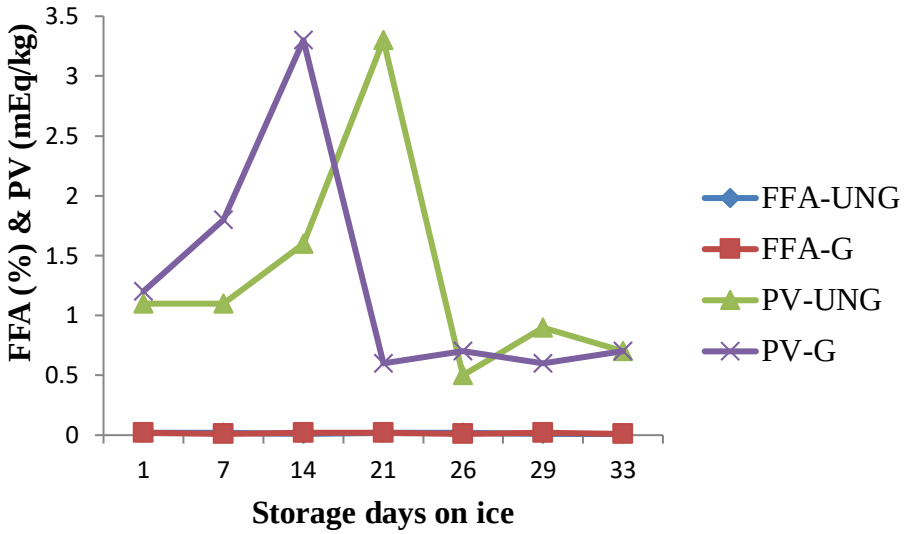

Figure 18: Free fatty acids and peroxide value trend over the storage days on ice for gutted and ungutted Nile perch

4.4 Correlation among the Sensory, Chemical and Microbiological Indices

The correlation among sensory, chemical and microbiological indices of the fish is shown in Table 3.

Table 3: Correlation among the sensory, chemical and microbiological indices

Indices	*QI Score*	*TVC(log cfu/g)*	*SSB (log cfu/g)*	*TVBN (mgN/100g)*	*FFAs (%)*	*PV (mEq/kg)*
QI Score	_	0.91	0.90	0.79	-0.40	-0.33
TVC (log cfu/g)	0.91	_	0.97	0.74	-0.42	-0.10
SSB (log cfu/g)	0.90	0.97	_	0.73	-0.38	-0.12
TVBN (mgN/100g)	0.79	0.74	0.73	_	-0.39	-0.36
FFAs (%)	-0.40	-0.42	-0.38	-0.39	_	-0.07
PV (mEq/kg)	-0.33	-0.10	-0.12	-0.36	-0.07	_

There were a positive correlation between QI score with TVC, SSB and TVBN, while other parameters such as FFAs and PV showed negative correlation at ($p<0.05$).

4.5 Sensory, Microbiological and Chemical Parameters for Nile Perch Sampled from the Cold Storage and Fish Factories

4.5.1 Sensory assessment

The average weight and length of the fish sampled from both sampling stations were 2 000 g and 53 cm, respectively. Fish from fish factories were ungutted while those from cold storage were gutted. The sensory assessment was done using the developed QIM scheme. The QI score was high ranged from 13.7-14.5 for factory A and B. Through sensory assessment we noted that, the texture of the flesh was very soft while other parameters like eye clarity, gills among others were moderate. Therefore, the texture was the main factor that caused rejection of these fish because they were not qualified to be processed for export market (Fig. 19).

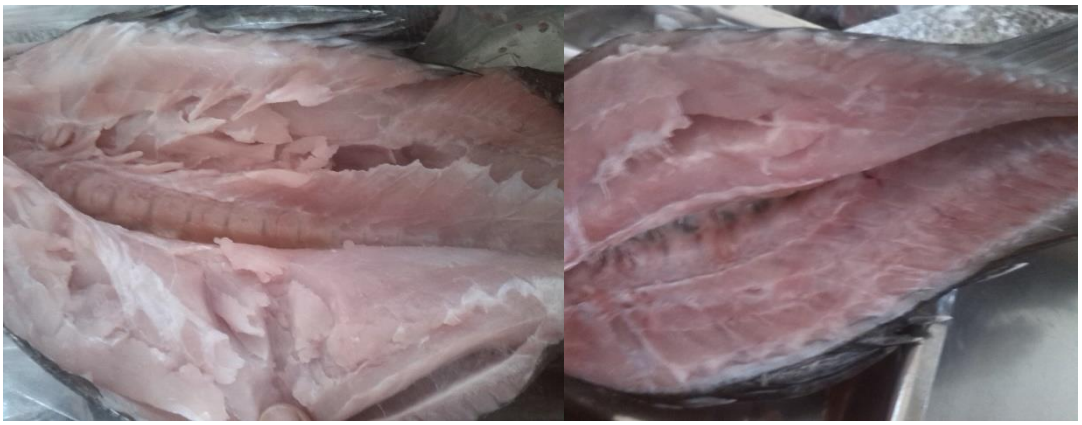

Figure 19: The appearance of the Nile perch flesh rejected from fish factories through sensory assessment. The one on the left side is from factory "A" and that the on the right side was from factory "B"

However, the Nile perch sampled from the three cold stores (C, D and E) was 1-4 weeks frozen and the average weight and length were the same as in fish factories. The QI score was low 4.8, 4.2 and 3.15 for cold storage C, D, and E, respectively. There were little changes observed on the gills, eyes, skin and odour among others through sensory

assessment. Generally, the Nile perch from the cold storage was organoleptically good compared to those from fish factories.

4.5.2 Total viable counts and specific spoilage bacteria

The Total Viable Counts and Specific Spoilage Bacteria were high for the rejected Nile perch samples from the fish factories compared to Nile perch samples from the cold storage. The TVC was 5.21 and 5.05 $\log_{10}$ cfu/g while the SSB was 4.72 and 4.62 $\log_{10}$ cfu/g in factory A and B, respectively. The TVC ranged from 1.82 and 3.92 $\log_{10}$ cfu/g while the SSB ranged from 1.89-2.68 $\log_{10}$ cfu/g for samples from cold stores.

4.5.3 Total volatile basic nitrogen, free fatty acids and peroxide value

The TVBN, FFAs and PV were almost the same from all the sampling stations. The level of TVBN was 6.89 and 7.59 mgN/100 g for fish factories A and B, respectively. For the cold storage C, D and E the TVBN level were 7.96, 6.19 and 5.59 mgN/100 g, respectively. The FFAs ranged from 0.01-0.02% and the PV ranged from 0.55- 0.69 mEq/kg for all sampling stations. The means score for sensory assessment, TVC, SSB, FFAs and PV are presented in Fig. 20.

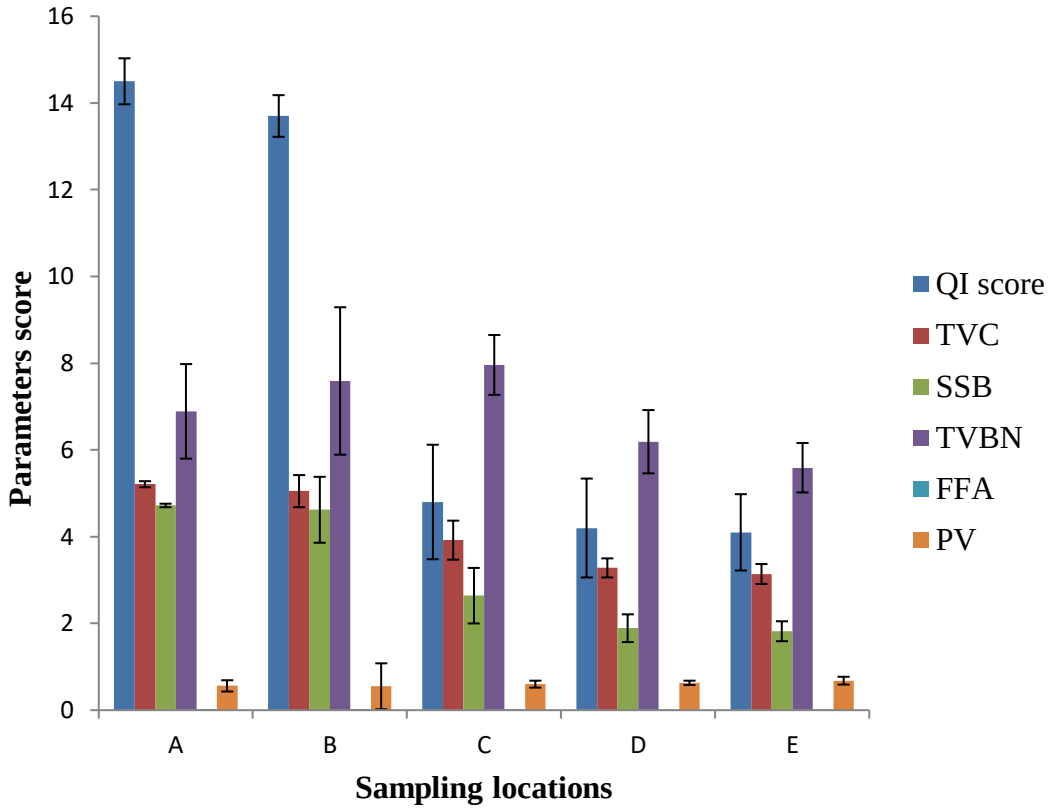

Figure 20: The overall mean scores for sensory, microbiological and chemical parameters for Nile perch samples from fish factories and cold storage. The mean score for Quality Index, Total Viable Count and Specific Spoilage Bacteria were higher for Nile perch from Fish factories compared to cold storage

CHAPTER FIVE

5.0 DISCUSSION

5.1 Sensory Analysis

5.1.1 The QIM scheme

The total demerit points in the developed QIM scheme were 23 and 24 for gutted and ungutted Nile perch, respectively. The current results were different from the one developed by Okeyo (2009) who got total demerit points of 20 for Nile perch in Kenyan side of Lake Victoria. There are some sensory attributes that the author did not include in the QIM. These included scales and smell in the general appearance which are among the important attributed to be evaluated. These parameters are also important in evaluation of fish because as the fish deteriorate, scales starts to loose from the skin, also fishy smell changed to off odour. Also he did not include pale red/slightly discoloured which is among the quality attribute used to evaluate the colour change in the gills. Also, the QIM that used by others like Mhongole (2009) and the modified from Larsen *et al.* (1992) to assess the freshness quality of Nile perch had the total demerit points of 20. Here, the author did not include slime and scales but had sensory attribute, which was black spots on the gill cover. In the present study, these were not included because none of the fish were found with such attribute. The current developed QIM allows direct establishment of criteria to accept or reject the fish product when needed for consumption or later transportation and processing because it determines the loss of freshness based on storage time. Also, it will be as tool to aid consumers to decide on the deterioration level of Nile perch according to the analysis of attributes described in the QIM for storage conditions on ice.

5.1.2 Shelf life study

The shelf life study for both gutted and ungutted Nile perch on ice was performed purposely to validate the QIM. During the shelf life study, quality parameters such as gills colour, skin, eye clarity, texture, and odour increased with storage time and were described

as the main contributors in freshness changes during ice storage of Nile perch. Other parameters such as eye shape, slime and belly flap fluctuated with storage days and were described as a minor contributors in freshness changes during ice storage of Nile perch. The QI scores increased with storage time on ice. This is due to loss of freshness, which eventually led to deterioration of quality. As the score increased, the freshness quality decreased, which made the fish be rejected by the panelists. There was high positive correlation between the QI score and the storage days ($r = 0.97$, $p<0.05$). This indicates that the QI score closely reflected the deteriorative changes in Nile perch with storage time and even predicted the remaining shelf life of fish on ice.

Martinsdottir *et al*. (2001) suggested that when iced fish is evaluated after catch, it should be scored low and the scores consequently increase with storage time, reaching close to maximum score at the end of the shelf life. Huss (1995) reported that when the QI score equals to 10 the remaining storage time on ice is estimated to be about 5 days. In this study the total QI score was 10 when the Nile perch storage time in ice was 14 days (Fig. 8). Therefore, it is suggested that the excellent freshness quality of Nile perch stored on ice should not be more than 2 weeks (14 days). Also, it is in agreement with Mhongole (2009), who suggested that the excellent freshness of whole Nile perch grade is 15 days (2 weeks) and good quality is between 20-28 days (3-4 weeks) of ice storage.

5.1.3 Sensory evaluation of cooked Nile perch

The acceptability for the odour, flavour and texture during the sensory evaluation of Nile perch significantly decreased ($p<0.05$) with storage time in ice from day 14 onwards. The high scores (10-8) observed on day 1-14 of storage showed the odour, texture and flavour of the cooked fish was fresh to sea weedy, elastic, sweet with a characteristic fish flavour respectively (Fig. 12). The sensory rejection point of ≤ 4 was characterized by traces of

slight off odours, bitter and sour flavour. According to the results, flavour and odour were selected to determine the rejection point of the cooked Nile perch samples at day 29 of storage, the point at which the cooked fillets were judged unacceptable. However, the parameters such as gill colour and odour, eye clarity and skin also reached the maximum demerit points of 3, 2.5 and 2, respectively.

The changes of odour, texture and flavour can have direct link with the TVC and SSB because as the storage days increased, the TVC and SSB also showed the same trend. These results were in agreement with Okeyo (2009), who suggested that the development of these sensory characteristics can be linked with the increase in the level of TVC and H_2S producing bacteria. These bacteria may have been responsible for the breakdown of non-protein compounds in the muscle to volatile amines and accumulation of FFAs. Also, Quang (2005) reported that bacteria (spoilage bacteria) use their enzyme to change fish odour and flavour to sour, gassy, fruity and finally ammonia and fecal odour.

5.2 Microbiological Analysis

The results (Fig. 13 and 14), give clear interpretation that as the QI score increases with storage time, the TVC and SSB also increase meaning that the sensory quality changes can be caused by spoilage bacteria. However, the TVC and SSB counts from day 21-33 of storage were beyond the acceptable limit set by TZS 402 (1988) standards for fish and fishery products specifications. The psychrotrophic count is 10^5 cfu/g or 5 $\log_{10}$ cfu/g thus, beyond that level the fish is considered unacceptable. Therefore, the use of the QIM can help the consumers/processors to judge the quality of fish before buying/ processing. However, there was a strong positive relationships between QI score and TVC ($r=0.91$) as well as for SSB ($r=0.97$) at $p<0.05$.

The results of TVC and SSB from this study almost resembled those obtained by Mhongole (2009) who found the SSB and TVC of less than 10 log cfu/g at the end of storage time (33 days). The increase in H_2S producing bacteria coincided with the onset of the advanced spoilage stage which the fish became unfit for human consumption, as proved by the unfavourable sensory scores given by the panelists on cooked flesh. Lokuruka (2015) reported that, gutting do not influence the variation in the hydrogen sulphide producing bacterial counts because it depends on environment where the fish was caught, species, age, fishing methods used and the fat content in the fish muscles. The isolated bacterial flora (black colonies), which were considered as SSB were oxidase and catalase positive, that they might be *S. putrefaciens* and /or some of *vibrionaceae* because they were able to produce H_2S from the sulphur-containing amino-acid L-cysteine. It has been also reported by Gram *et al.* (1987) that *S. putrefaciens* and *vibrionaceae* constituted a major fraction of the bacterial flora in Nile perch during ice storage at 0°C. The presence of these spoilage bacteria in fish stored on ice at 0°C agrees with previous studies by Gram *et al.* (1990) and Huss (1994).

5.3 Chemical Analysis

5.3.1 Total volatile basic nitrogen

The level of TVBN for both gutted and ungutted Nile perch increased with QI score as well as storage days. However, there was significant ($p<0.05$) positive correlation between QI score and TVBN ($r=0.79$). This significant increase coincided with the onset of spoilage and the logarithmic phase of microbial growth. The microbial levels may have been responsible for the breakdown of nitrogenous compounds resulting in volatile compounds hence an increase in the level of TVBN. The low level of TVBN revealed that it is not a good indicator to assess the freshness quality of Nile perch as compared to QI score and SSB. These results agreed with those by Mhongole (2009) who reported the

TVBN level of 17 mgN/100 g of whole Nile perch flesh in 33 days of storage on ice. According to ICMSF (1998), these levels are very low compared to other species which may have TVB-N in the range of 20-30 mgN/100 g during the first 2 weeks and > 30 mg/100 g after 2 weeks of storage on ice. Also, it is in agreement with Massete (1999), who reported TVBN level >20 mgN/100 g in Nile perch at the end of storage time (19 days) on ice. On the other hand, these results disagree with those by Okeyo (2009) who reported the level of TVBN for ungutted and gutted Nile perch to be 26.03 and 24.52 mgN/100 g on day 26 and 28 of storage, respectively, whereby cooked fish considered unfit for human consumption.

Lokuruka (2015) suggested the reject grade for farmed tilapia with the TVBN level of 25 mgN/100 g on day 30 for the ungutted and day 34 for the gutted. Karungi *et al.* (2004) reported that the TVBN contents in fresh Nile perch varied between 9 and 11 mgN/100g and reached up to 28 mgN/100g after 20 days in ice. TVBN includes the measurement of trimethylamine (TMA) produced by spoilage bacteria such as *Pseudomonas spp.* and *S. putrifaciens,* dimethylamine (DMA) produced by autolytic enzymes during frozen storage and ammonia produced by the deamination of amino acids and nucleotide catabolites (Huss, 1995). TVBN is considered to reflect only stages of advanced spoilage in fish and is considered unreliable for the measurement of spoilage during the first ten days of ice storage of fish. However, TVBN values do identify the later stages of spoilage and therefore can be used as a routine method to determine if chilled seafood is spoiled (Sykes *et al*., 2009).

Also, it can be suggested that the level of TVBN vary depending on the species, environment and season. The marine fish species contain high TMO compared to freshwater fish species and also different fresh water species have different TVBN levels.

The presence of TVBN in fish can be taken as an indication of bacterial growth, while the ammonia comes from decomposition of amino acids, thus reducing the quality of the available protein (Jinadasa, 2014). TVBN is insensitive to freshness, which means that it cannot be used as a freshness indicator but it is sensitive in terms of fitness for human consumption and it is also a good spoilage indicator (Shakhtour and Yesim, 2000). Therefore, the low level of TVBN found in this study can indicate that there is much less TMAO and H_2S-forming bacteria in Nile perch that relied on compounds other than TMAO as electron acceptors (Massete, 1999).

5.3.2 Free fatty acids

The level of FFAs of both gutted and ungutted Nile perch was the same from day 1 up to the end of the storage time (33 days) in ice. There was no linear relationship between the FFAs and QI as well as storage days. The correlation between FFA and QI score was significant but with negative relationship ($r = -0.40$, $p<0.05$). The results from this study are disagree with those from Lokuruka (2015) who found 2.4-2.6 mg FFAs/100g at the end of storage time (25 days) in Nile perch at the point where the fish was rejected by the panelists. Also, Okeyo (2009) reported the FFAs content of 2.59% on day 26 for the gutted fish samples from the landing sites and FFAs content of 2.65% on day 28 of storage in ice for freshly caught and gutted Nile perch, which were considered unfit for human consumption. Other authors Tenyang *et al.* (2017) reported the increase of acid value/FFAs up to 6.17% in catfish stored in refrigerator for 9 days. FFAs can undergo further oxidation to produce compounds with low molecular weight, which are responsible for the rancidity of fish and fish products (Fennema, 1996). According to Aryee and Simpson 2009, the FFAs measure the extent of decomposition of lipase action. Therefore, the low FFAs value obtained in this study revealed that the lipase content was low.

5.3.3 Peroxide value

During storage, significant decrease was observed in the PV. The PV for day 1 was 1.2 mEq/kg of lipid then increased rapidly to 3.3 mEq/kg of lipid in day 21 and finally dropped to 0.7 mEq/kg of lipid in 33 days of storage. There was a negative correlation with the QI score ($r=-0.33$, $p<0.05$). Therefore, according to the results obtained in this study, lipid oxidation does not appear to be a dominant spoilage process in Nile perch stored in ice. The results are in agreement with those obtained by Smith *et al.* (1980, 1980a) that in wet fish storage, components introduced primarily by bacterial spoilage as well as by enzymatic reactions contribute more to the flavour than those derived from lipid autoxidation. Also Fennema (1996) reported that the decrease in PV value confirms that during storage, the components formed are unstable and highly susceptible to further changes. They are rapidly transformed into various volatile and nonvolatile compounds such as aldehydes, ketones and acids.

Also Tenyang *et al*. (2017) found a decrease of PV when catfish was stored in a refrigerator for 9 days. The other study conducted by Shakhtour *et al.* (2013) showed rapid fluctuation of PV in tilapia fillets during ice storage from 12.58 mEq/ kg of lipid and decreased suddenly to 4.16 mEq/kg of lipid at the end of storage. The author suggested that the slight decrease in PV at the end of storage could be due to the fact that hydroperoxides formed might decompose into other compounds. Lipid hydroperoxides are formed by various pathways, including the reaction of singlet oxygen with unsaturated lipids or the lipoxygenase-catalyzed oxidation of polyunsaturated fatty acids. However, the results is disagree with other authors like Peng *et al*. (2016) who suggested that the cold storage duration increases the production of secondary products of lipid oxidation. Also Pacheco-Anguilar *et al*. (2000) reported an increase in PV in oily Monterey sardines stored at 0˚C for 15 days. The PV is used to estimate the initial evidence of rancidity,

which occurs by autoxidation in unsaturated fats and oil. So the oxidation reaction can decrease the nutritional quality of food/fish. Therefore, the low PV means the ability of the fats or oil to go rancid is also low (Othman and Ngassapa, 2010). The variation of PV may be due to the storage temperature, the concentration of pro-oxidants, enzymes, ionic strength and oxygen consumption (Bustabad, 1999).

5.4 Sensory, Microbiological and Chemical Parameters for Nile Perch Sampled from the Cold Storage and Fish Factories

Generally, the quality of Nile perch sampled from the fish factories in terms of was not so bad organoleptically but the drawback was in texture. Other parameters such as eye clarity, gills colour among others were moderate. The texture of the muscle was very soft and it was one of the factors that caused the rejection of these fish because such kind of fillet cannot be processed for export. This could be due to autolytic changes that occur in fish muscles during ice storage. Nile perch sampled from cold storage were organoleptically good. However, the TVC and SSB were high for the Nile perch sampled from fish factories compared to samples collected from cold storage. This was because the QI score had a direct relationship with TVC and SSB. These variations could be due to different storage temperatures, failure to replace the ice once it melted and may be the handling methods. The samples from the cold storage were gutted and frozen at -15ºC while from fish factories were ungutted and ice chilled. However, the results are in agreement with Kaale *et al.* (2011) who reported that storing the fish at super chilling temperature can maintain the food freshness, retaining high food quality and suppressing growth of spoilage bacteria.

Also, Duun and Rustad (2008) reported that super chilling of salmon stored at -1.4 to -3.6˚C doubled the shelf life compared to ice chilled storage with respect to microbial and

chemical parameters. Factors such as the age of the fish, the species, the amount of lipid, the catching ground and method of catch determine whether gutting of fish could be advantageous or not (Huss, 1988). There was a little variation of TVBN levels while the FFAs and PV were almost the same for all sampling stations. As per quality specifications for crude fish oil, maximum acceptable values of FFAs is 2-5% and acceptable level of peroxide value for human consumption should not exceed 20 mEq/kg of lipid (Bimbo, 1998 and Bako, 2014). The acceptable limit of TVC in fish is 10^1 - 10^5 cfu/g while for SSB is 10^1 - 10^2 cfu/g according to Tanzania standard. Therefore, beyond that level the fish is considered unacceptable. Generally, fish from fish factories were not accepted organoletically because texture covered all impressions when food comes into contact with human surfaces example finger, tongue or teeth. According to Munoz *et al.* (2002), sensory analysis plays an important role in quality control and quality assurance in the fish sector. Other parameters such as microbiological and chemical were within acceptable limit set by TZS 402 (1988) standard for fish and fishery products specifications.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The major sensory attributes of the QIM scheme were gills colour, skin colour, odour, texture and eye clarity so the consumers should use these attributes in judging the freshness of fish before purchasing. The good freshness quality Nile perch is the one stored on ice for not more than two weeks (14 days), which results in QI score of 10 or less. In addition, parameters such as QI score, TVC and SSB shows linear relationship with the storage time hence these parameters are suggested to be good indicators for studying the shelf life of Nile perch stored on ice. Whereas other parameters such as TVBN, FFAs and PV were not good indicators for shelf life study of Nile perch stored on ice because they did not reach the maximum limit at the end of storage time. The overall quality of fish from fish factories was not good organoleptically but acceptable in microbiological and chemical parameters. However, the adoption of the QIM scheme will assist the fishermen to judge the quality and handling the fish on board and assist inspection of condition of fish by consumers, inspectors and regulation authorities.

6.2 Recommendations

i. Consumers and fish inspectors need to use the QIM scheme in deciding the freshness and /or deterioration level of Nile perch.

ii. For good sensory and microbiological quality, Nile perch should not be stored on ice for more than two weeks.

iii. In purchasing Nile perch, appearance of the gills colour, skin, texture, eye clarity and odour should be used to judge freshness.

iv. For good quality product, fish processing plants should control the freshness stage of their raw materials and their storage.

v. Since the study was done on the weekly basis, there was difficulty in telling exactly when spoilage occurred. Therefore, similar studies need to be conducted to establish the optimum shelf life by sampling daily.

vi. As aquaculture sector is growing rapidly, further studies to develop QIM scheme for farmed fish species in Tanzania especially Tilapia (*Oreochromis niloticus*) and Catfish (*Clarias gariepinus*) is highly recommended.

REFERENCES

Abbas, K. A., Mohamed, A., Jamilah, B. and Ebrahimian, M. (2008). A review on correlations between fish freshness and pH during cold storage. *American Journal of Biochemistry and Biotechnology* 4(4): 416 - 421.

Adams, M. R and Moss, M. O. (2008). *Food Microbiology*, Third Edition. The Royal Society of Chemistry. Thomas Graham House, Science Park, Milton Road, Cambridge CB4, UK. 463pp.

Adawiyah, D. R., Soekarto, T. S. and Hariyadi, P. (2012). Fat hydrolysis in a food model system: effect of water activity and glass transition. *International Food Research Journal* 19(2): 737 - 741.

Amegovu, A. K., Serunjogi, M. L., Ogwok, P. and Makokha, V. (2012). Nucleotide degradation products, total volatile basic nitrogen, sensory and microbiological quality of Nile pearch (*Lates niloticus*) fillets under chilled storage. *Journal of Microbiology, Biotechnology and Food Sciences* 2(2): 653 - 666.

Aryee, A. N. A. and Simposon, B. K. (2009).Comparative studies on the yield and quality of solvent extracted oil from salmon skin. *Journal of food Engineering* 92(3): 353-358.

Azhar, K. F. and Nasa, K. (2006). Lipid and their oxidation in seafoods. *Journal Chemistry and Society* 28(3): 298 - 303.

Baixas-Nogueras, S., Bover-Cid, S., Veciana-Nogués, T., Nunes, M. L. and Vidal-Carou, M. C. (2003). Development of a Quality Index Method to evaluate freshness in

mediterranean hake (*Merluccius merluccius*). *Journal of Food Science* 68(3): 1067-1071.

Bako, T., Unogbai, V. I. and Obetta, S. E. (2014). Extraction and characterization of Mackerel (*Scomber scombrus*) oil for industrial use. *Researcher* 6(8): 80 - 85.

Bernárdez, M., Pastoriza, L., Sampedro, G., Herrera, J. J. and Cabo, M. L. (2005). Modified method for the analysis of free fatty acids in fish. *Journal of Agricultural and Food Chemistry* 53(6): 1903-1906.

Bernardi, D. C., Másrico, E. T. and Freitas, M. Q. D. (2013). Quality Index Method (QIM) to assess the freshness and shelf life of fish. *Brazilian Archives of Biology and Technology* 56(4): 587 - 598.

Bimbo, A. P. (1998). Guidelines for Characterizing Food-Grade Fish Oils. International fishmeal and oil manufacturers association (IFOMA). pp 473 - 483.

Bligh, E. G. and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Journal of Biochemical Physiology* 37(8): 911 - 917.

Botta, J. R. (1995). *Evaluation of Seafood Freshness Quality*. VCH Publishers Inc. New York. 180pp.

Bremner, H. A. and Hallett, I. C. (1985). Muscle fiber-connective tissue junctions in the blue grenadier (*Macruronus novaezelandiae*). A scanning electron microscope study. *Journal of Food Science* 50(4): 975 - 980.

Bustabad, O. M. (1999). Weight loss during freezing and the storage of frozen meat. *Journal of Food Engineering* 41(1): 1-11.

Chytiri, S., Chouliara, I., Savvaidis, I. N. and Kontominas, M. G. (2004). Microbiological, chemical and sensory assessment of iced whole and filleted aquacultured rainbow trout. *Food Microbiology* 21(2): 157-165.

Coletta, J. M., Bell, S. J. and Roman, A. S. (2010). Omega-3 fatty acids and pregnancy. *Reviews in Obstetrics and Gynecology* 3(4): 163-171.

Connell, J. J. (1990). *Control of Fish Quality,* 3rd ed. Oxford, Aitken: Blackwell Publishers. 256pp.

Dalgaars, P. (2000). *Fresh and Lightly Preserved Seafood; Shelf-life Evaluation of Foods.* 2nd ed. London: Aspen Publishers, Inc. pp110 - 139.

David, O. M., Wandili, S., Kakai, R. and Waindi, E. N. (2008). Isolation of *Salmonella* and *Shigella* from fish harvested from the Winam Gulf of Lake Victoria, Kenya. *Journal of Infect Developing Countries* 3(2): 99 - 104.

Dergal, N. B., Abi-Ayad, S. M. E. A., Douny, C., Brose, F., Daube, G., Rodrigues, A. and Scippo, M. L. (2013). Microbial, biochemical and sensorial quality assessment of Algerian farmed tilapia (*Oreochromis niloticus*) stored at 4 and 30˚C. *African Journal of Food Science* 7(12): 498 - 507.

Duun, A. S. and Rustad, T. (2008). Quality of super chilled vacuum packed Atlantic salmon (*Salmo salar*) fillets stored at− 1.4 and− 3.6 C. *Food Chemistry* 106(1): 122-131.

Egan, H., Kirk, R. S. and Sawyer, R. (1981). Oil and Fat: *in Pearson's Chemical Analysis of Foods*. New York: Longman Scientific and Technical Press. 536pp.

FAO, Food and Agriculture Organization of United Nations (2013). The state of world aquaculture and fisheries, 2013. FAO Fisheries and Aquaculture Department, Rome. pp 157-160

Fennema, O. R. (1996). *Food Chemistry*, Marcel Dekker. *Inc., New York*. pp 225 - 319.

Gram, L. (1990). Spoilage of three Senegalese fish species stored in ice and at ambient temperature. Paper presented at SEAFOOD 2000 in Halifax, Canada. pp 12 - 16.

Gram, L., Trolle, G. and Huss, L.L. (1987). Detection of specific spoilage bacteria on fish stored at high (20ºC) and low (0ºC) temperatures. *International Journal of Food Microbiology* 4(1): 65-72.

Horsfall, M., Kinigoma, B. S. and Spiff, A. I. (2006). Evaluation of the levels of total volatile bases and trimethylamine formed in fish stored at low temperature. *Chemical Society of Ethiopia* 20(1): 155-159.

Howgate, P. F. (1982). Quality assessment and quality control. *Fish handling and processing*. pp 177 - 186.

Huis, J. H. (1996). Microbial and biochemical spoilage of foods: an overview. *International Journal of Food Microbiology* 33(1): 1-18.

Huss, H. H. (1988). *Fresh fish quality and quality changes: a training manual prepared for the FAO/DANIDA Training Programme on Fish Technology and Quality Control* (No. 29). Food and Agriculture Organization.132pp.

Huss, H. H. (1994). *Assurance of Seafood Quality*. FAO Fisheries Technical paper No: 334, Roma. 169pp.

Huss, H. H. (1995). *Quality and Quality Changes in Fresh Fish*. FAO fisheries technical paper 348, Rome. 195pp.

Hyldig, G. and Green-Petersen, D. M. (2005). Quality Index Method—an objective tool for determination of sensory quality. *Journal of Aquatic Food Product Technology* 13(4): 71 - 80.

Hyldig, G. and Nielsen, D. (2001). A review of sensory and instrumental methods used to evaluate the texture of fish muscle. *Journal of Texture Studies* 32(3): 219 - 242.

ICMSF, International Commission on Microbiological Specification for Food (1998). International Commission on Microbiological Specification for Food: *Microbial Ecology of Food Commodities*. (Vol.6) London: Blackie Academic and Professional. pp 159 - 173.

ISO, International Organization for Standardization 4833: 2003 (E). Enumeration of Microorganisms-Colony Count Technique at 30^{o}C. 7pp.

ISO, International Organization for Standardization 8586-1:1993. Sensory analysis− General guidance for the selection, training and monitoring of assessors - Part 1: Selected assessors. 67pp.

ISO, International Organization for Standardization 8589:2007. Sensory analysis - General guidance for the design of test rooms. 67pp.

Jinadasa, B. K. K. K. (2014). Determination of quality of marine fishes based on Total Volatile Base Nitrogen test (TVB-N). *Nature and Science* 5(12): 106 - 111.

Jorge, R.J., Redondo, P.N., Dosta, M. and Del, C. M. (2016). Bacterial load comparison of marine fish collected and commercially obtained for human consumption in western region of Yucatan Peninsula, Mexico. *International Journal of Aquatic Science* 7(1): 6 - 12.

Jørgensen, B. R., Gibson, D. M. and Huss, H. H. (1988). Microbiological quality and shelf life prediction of chilled fish. *International Journal of Food Microbiology* 6(4): 295-307.

Kaale, L. D., Eikevik, T. M., Rustad, T. and Kolsaker, K. (2011). Super chilling of food: a review. *Journal of Food Engineering* 107(2): 141 - 146.

Kanner, J. (1994). Oxidative processes in meat and meat products: quality implications. *Meat Science* 36(1): 169-189.

Karungi, C., Byaruhanga, Y.B. and Muyonga, J.H. (2004). Effect of pre-icing duration on quality deterioration of iced Nile perch (*Lates niloticus*). *Food Chemistry* 85 (1): 13 - 17.

Kirk, S. and Sawyer, R. (1991). *Pearson's Composition and Analysis of Foods* (9th Ed). Longman Group Ltd. London. 708pp.

Kovacs, N. (1956). Identification of Pseudomonas pyocyanea by the oxidase reaction. *Nature* 178(4535): 703-709.

Kussaga, J. B., Luning, P. A., Tiisekwa, B. P. M. and Jacxsens, L. (2014). Challenges in performance of food safety management systems: a case of fish processing companies in Tanzania. *Journal of Food Protection* 77(4): 621-630.

Kyangwa, I. and Odongkara, K. (2005). Sanitation, fish handling and artisanal fish processing within fishing communities: Socio-cultural influences. LVEMP Socio-economic Research Report 6. NARO-FIRRI, Uganda. 46pp.

Larsen, E., Heldbo, J., Jespersen, C. M. and Nielsen, J. (1992). Development of a method for quality assessment of fish for human consumption based on sensory evaluation. *Developments in Food Science*. pp 351-358.

Lokuruka, M. N. (2015). Biochemical and microbiological indices for a Kenya Standard for fresh finfish. *Egerton Journal of Science and Technology* 15: 11 - 29.

Mahesar, S. A., Sherazi, S. T. H., Khaskheli, A. R. and Kandhro, A. A. (2014). Analytical approaches for the assessment of free fatty acids in oils and fats. *Analytical Methods* 6(14): 4956-4963.

Malle, P. and Poumeyrol, M. (1989). A new chemical criterion for the quality control of fish: trimetylamine/total volatile basic nitrogen (%). *Journal of Food Protection* 52(6): 419 – 423.

Martinsdóttir, E. (2002). Quality management of stored fish. In: Bremner, H.A. eds. *Safety and Quality Issues in Fish Processing*, England: Woodhead Publishing Limited. pp 360 – 378.

Martinsdottir, E., Sveinsdottir, K., Luten, J., Schelvis-Smit, R. and Hyldig, G. (2001). Reference manual for the fish sector: Sensory evaluation of fish freshness. QIM-Eurofish, AB IJumiden, the Netherlands. pp 1 - 60.

Masette, M. A. (1999). Comparative study of storage time of warm and cold water fish in view of the current market demand. *The United Nations University, Fisheries Training Programme*. Uganda 42pp.

Méndez-Bustabad, O. (1999). Weight loss during freezing and the storage of frozen meat. *Journal of Food Engineering* 41(1): 1 - 11.

Mhongole, O. J. (2009). Microbiology and Spoilage Trail in Nile Perch (*Lates niloticus*), Lake Victoria Tanzania. Dissertation award, MSc. degree at University of Iceland. Iceland. 94pp.

MOLFD, Ministry of Livestock and Fisheries Development (2013). Ministry of Livestock and Fisheries Development. Fisheries Annual Statistical Report.

Munoz, A. M. (2002). Sensory evaluation in quality control: an overview, new developments and future opportunities. *Food Quality and Preference* 13(6): 329-339.

Nguyen, M. V., Thorarinsdottir, K. A., Thorkelsson, G., Gudmundsdottir, A. and Arason, S. (2012). Influences of potassium ferrocyanide on lipid oxidation of salted cod (*Gadus morhua*) during processing, storage and rehydration. *Food Chemistry* 131(4): 1322 - 1331.

Nielsen, D. and Hyldig, G. (2004). Influence of handling procedures and biological factors on the QIM evaluation of whole herring (*Clupea harengus*). *Food Research International Journal* 37(10): 975 - 983.

Okeyo, G. O. (2009). Development of a Quality Index Method Scheme to Evaluate Freshness of Ice stored Lake Victoria Nile perch (*Lates niloticus*). Dissertation for Award, MSc. Degree at Egerton University, Kenya. 70pp.

Olafsdottir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., Mackie, I.M., Henehan, G., Nielsen, J. and Nilsen, H. (1997). Methods to evaluate fish freshness in research and industry. *Trends Food Science and Technology* 8(8): 258 - 265.

Olsen, S. O. (2004). Antecedents of seafood consumption behavior: An overview. *Journal of Aquatic Food Production and Technology* 13(3): 79 - 91.

Onjong, H. A., Wangoh J. and Njage, P. M. K. (2014). Current food safety management systems in fish-exporting companies require further improvements to adequately cope with contextual pressure: case study. *Journal of food Science* 79(10): 2031 - 2039.

Othman, O. C. and Ngassapa, F. N. (2010). Physical chemical characteristics of some imported edible vegetable oils and fat marketed in Dar es Salaam Tanzania. *Journal of Natural and Applied Sciences* 1(2): 138-147.

Özyurt, G., Kuley, E., Özkütük, S. and Özogul, F. (2009). Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. *Food chemistry* 114(2): 505-510.

Pacheco-Aguilar, R., Lugo-Sanchez, M. E. and Roblos-Bugueno, M. R. (2000). Postmortem biochemical and functional characteristics of Monterey sardine muscle stored at 0ºC. *Journal of Food Science* 65(1): 40 - 47.

Peng, L., Hua, Y., Yingchun, Z., Yang, W., Dongqing, B., Ruitong, D., Xiaoqing, R., Hongshun, Y. and Lizhen, M. (2016). Influence of washing and cold storage on lipid and protein oxidation in catfish (*Clarias lazera*) surimi. *Journal of Aquatic Food Production* 25(6): 790 - 800.

Quang, N. Y. (2005). Guidelines for handling and preservation of fresh fish for further processing in Vietnam. *The United Nations University, Fisheries Training Programme*. Iceland. 57pp.

Shahidi, F. and Zhong, Y. (2005). Antioxidants: regulatory status. *Bailey's industrial oil and fat products*. pp 491-512.

Shakhtour, F. G. M. and Babji, A. S. (2013). The effect of powdered cumin sprinkling on biochemical and quality attributes of red tilapia fillets stored in ice. *Journal of Food Technology* 11(2): 14 - 21.

Smith, J. S. M., Hardy, R., MacDonald, I. and Templeton, J. (1980). The storage of herring (*clupea harengus*) in ice, refrigerated sea water and at ambient temperature. Chemical and sensory assessment. *Journal of the Science of Food and Agriculture* 31(4): 375 - 385.

Sveinsdottir, K., Hyldig, G., Martinsdottir, E., Jorgensen, B. and Kristbergsson, K. (2003). Quality Index Method (QIM) scheme developed for farmed Atlantic salmon (*Salmo salar*). *Food Quality and Preference* 14(3): 237 – 245.

Sykes, A. V., Oliveira, A. R., Domingues, P. M., Cardoso, C. M., Andrade, J. P. and Nunes, M. L. (2009). Assessment of European cuttlefish (*Sepia officinalis*, L.) nutritional value and freshness under ice storage using a developed Quality

Index Method (QIM) and biochemical methods. *Food Science and Technology* 42(1): 424-432.

TZS, Tanzania Standards, (1988). Fish and Fish Products-Microbiology Specifications. TZS 402: 1988 ICS 65. 150.

Tenyang, N., Womeni, H. M., Tiencheu, B., Villeneuve, P. and Linder, M. (2017). Effect of refrigeration time on the lipid oxidation and fatty acid profiles of catfish (*Arius maculatus*) commercialized in Cameroon. *Grasas y Aceites* 68(1): 177-186.

Venugopal, V. (2002). Biosensors in fish production and quality control. *Biosensors and Bioelectronics* 17(3): 147 - 157.

Wanasundara, U. N., Shahidi, F. and Amarowicz, R. (1998). Effect of processing on constituents and oxidative stability of marine oils. *Journal of Food Lipids* 5(1): 29 - 41.

Wu, T. H. and Bechtel, P. J. (2008). Ammonia, dimethylamine, trimethylamine, and trimethylamine oxide from raw and processed fish by-products. *Journal of Aquatic Food Product Technology* 17(1): 27-38.

APPENDIX

Appendix 1: Evaluation form for cooked Nile perch muscles using structure acceptability scale adapted from Huss (1995)

Your Sex…….Occupation…….

Instructions

Choose the best description to characterize the code. Write the corresponding value in the column to the right for the description under the right code. Please note the choice between two sets of description for each of the three parameters

Odour	Flavor	Texture	Score	Fish code	Comment(s) if any
Species specific, fresh fish	Typical of Nile perch flavor, sweet, watery	Firm, elastic	10		
Typical of Nile perch fresh	Sweet, fishy flavour	Less firm, easy to chew and swallow	9&8		
Neutral, Slightly fishy	Neutral, slightly fish flavor	Mealy & succulent initially	7&6		
Sour, stale, cabbage	Musty, fishy, slightly sour, some slightly off-flavour	Soft, mealy	5&4		
Rotten, spoilt stinking, strong ammonia like	Rotten, sour	Very soft, slippery,	3,2 &1		
Quality score					