DEVELOPMENT OF A QUALITY INDEX METHOD (QIM) SCHEME TO EVALUATE FRESHNESS OF ICE STORED LAKE VICTORIA NILE PERCH (*Lates niloticus*)

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DECLARATION AND APPROVAL

DECLARATION

This thesis is my original work and has not been presented in any other institution/university for the award of any certificate, diploma or degree.

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This thesis is dedicated to my mother Alice Okeyo, my wife Beverline Otieno, my children John Bruno and Talia Blessings and my late father John Okeyo.

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ABSTRACT

Despite the Nile perch contributing about 67% of Kenyaôs total annual fish export earnings, no specific method exists for evaluating the freshness of the fish when landed and its shelf life when kept in ice. Currently the European Union (EU) sensory scheme is used in sensory evaluation of all commercial fish species available in the Kenyan fishery. This study was conducted to develop a suitable Quality Index Method scheme, that is specific for the Nile perch, using a set of selected sensory parameters to help follow its deterioration profile in order to estimate changes in its freshness in the course of storage in ice. Fish samples for the study were obtained from the beaches. The fish samples from each location were divided into two groups; ungutted and gutted. The selected sensory parameters were correlated with selected biochemical and microbiological parameters. Significant increase ($p<0.05$) in the levels of sensory, microbiological and chemical parameters was observed on the 10^{th} and 14^{th} day onwards for the ungutted experimental and control samples, respectively and on the 14^{th} and 18^{th} day for the gutted experimental and control fish samples, respectively. The Quality index scores had a correlation of 0.98 with H_2S producing bacteria, 0.93 with total viable counts (TVC), 0.97 with total volatile basic nitrogen (TVBN), 0.96 with free fatty acids (FFA) and 0.97 with pH. The ungutted fish from the beaches and fishing ground had a shelf life of 22 and 28 days, respectively. The gutted fish samples from the beaches and fishing grounds had a shelf life of 26 and more than 28 days in ice, respectively. Gutting extended the shelf life of the Nile perch by about 4 days for both the beach-purchased and controls. The microbial, sensory and chemical parameters levels increased with increase in storage days both for ungutted and gutted fish samples from the beaches and fishing ground. The protein content decreased with increasing storage time in the ungutted experimental and control samples than in the gutted fish samples. There was no significant variation ($p>0.05$) in the moisture, lipid and ash content for both ungutted and gutted fish samples. The study is useful setting standards and guidelines which will be used by the Fish Inspectors in the enforcement of sensory quality of fish destined for the domestic and export markets. This will prevent occasional interruptions of exports to the European Union markets thereby minimizing the resultant huge financial losses to the industry hence creating steady employment to the locals.

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LIST OF ABBREVIATIONS

AOAC- Association of Official Analytical Chemists

BGA- Brilliant Green Agar

BGLB- Brilliant Green Lactose Bile

DMA- Dimethylamine

EEC- European Economic Commission

EU- European Union

FDA- Food and Drug Administration

FFA- Free Fatty Acids

GOK- Government of Kenya

H_2S- Hydrogen Sulphide

ICMSF- International Commission on Microbiological Specifications for Food

IMP- Inosine Monophosphate

IMVIC- Indole, Methyl red, Voges-proskauer and Citrate test

KEBS- Kenya Bureau of Standards

L-EMB- Levinesô Eosin-Methylene Blue Agar

NPN- Non Protein Nitrogen

QDA- Quantitative Descriptive Analysis

QI- Quality Index

QIM- Quality Index Method

TMAN- Trimethylamine Nitrogen

TMAO- Trimethylamine Oxide

TSI- Triple Sugar Iron

TVBN- Total Volatile Basic Nitrogen

TVC- Total Viable Count

VRBLA- Violet Red Bile Lactose Agar

XLD- Xylose lysine desoxycolate

CHAPTER ONE
INTRODUCTION

1.1 Background

In Kenya, the fisheries sub-sector is of great socio-economic importance, especially in the Lake Victoria basin. The sub-sector supports 80,000 fishers directly and 800,000 individuals indirectly (processors, traders and other service providers), through employment, income generation and export earnings to the riparian communities (GOK, 2003). The sub-sector is important in meeting the food security and nutritional requirements of riparian populations (Abila and Jansen, 1997). In the year 2006 through export, the sub-sector earned the country over Kshs. 4.9 billion (US $ 75 million) in foreign exchange from the export of 36,368 metric tons of fish and fishery products (Fisheries Department, 2006).

Nile perch (*Lates niloticus)* is among the most important commercial fish species from the Lake Victoria fishery. It is locally known as *'Mbuta'.* In the year 2005, the Nile perch production was ranked second in Kenya with 55,706 metric tons, after Omena (*Rastrineobola argentea)* with 57,929 metric tons while Nile tilapia (*Oreochromis niloticus*) was ranked third with 19,038 metric tons. The Nile perch export contributed Kshs. 3.3 billion, which is about 67% of the total export earnings from fish for the year 2005. However, the post harvest loss of this fish is 20 to 30 % annually (Fisheries Department, 2006).

1.2 Fish quality

Freshness is one of the aspects that determine the quality of fish and fishery products. Loss of freshness is a result of autolytic changes which involve enzymatic degradation of proteins to produce low molecular weight peptides and free amino acids (Gram and Huss, 1996). It is also due to the bacteriological changes which involve the growth of putrefactive bacteria e.g *Pseudomonas spp., Salmonella spp., Clostridium spp.*, which are able to degrade low molecular weight components to produce volatile metabolites. The loss in freshness may also be due to lipid oxidation and hydrolysis which result in the production of thiobarbituric acid reactive substances and free fatty acids (Huss, 1995). Feacal coliforms especially *Escherichia coli* are indicator organisms whose presence in foods indicate exposure to conditions that might allow the proliferation of enteric pathogenic organisms like *Salmonella* spp. Their presence or absence has value in assessing the safety and hygienic handling of foods. They also break down fish flesh nitrogenous components during metabolism to volatile nitrogenous compounds such as ammonia

which are responsible off flavour and this causes deterioration in the freshness of fish (Civera *et al*., 1993).

Currently freshness of fish is measured using sensory parameters using the European Union (EU) sensory scheme. The EU scheme describes characteristics such as the appearance of the skin, eyes, gills and flesh; the condition of the flesh, vertebral column and peritoneum; and the smell of the skin, gills and abdominal cavity. These parameters are then clustered in four quality levels E (extra), A (acceptable), B (poor) and C (unacceptable for human consumption) and they apply to all fish species. The Kenyan fishery relies on the European Union sensory evaluation scheme. The EU scheme has three weaknesses. First, it does not account for the differences between fish species because it assumes that the sensory characteristics described therein are common in every fish species. Secondly, it groups several different sensory terms into one quality grade and assumes that the parameters described have an equal influence on the rate of deterioration. Thirdly, the sensory characteristics of some species assessed using the scheme disagrees with other sub descriptions defined within the quality grade of the EU scheme.

Another method referred to as Quality Index Method (QIM) scheme has been developed as a sensory method where the descriptions of the individual grades are precise, objective and independent rather than using a cluster of terms. This scheme is based on scored detailed descriptors that are grouped into distinct and independent characters within general attributes such as skin surface appearance, skin stiffness, eyes clarity, eyes shape, color of gills and odor to evaluate the quality of fish. The scores range from 0 to1, 0 to 2 or 0 to 3 with 0 denoting fresh characteristic of a given attribute while 1, 2 and 3 denotes deterioration in the attribute being analysed.

The Nile perch species being an important foreign exchange earner, requires a specific method for evaluating its freshness when landed and at the various stages of its handling. This study was therefore designed to develop a specific method for assessing the freshness of Nile perch species based on the sensory, chemical and microbiological changes as a basis for establishing a set of guidelines for the proper handling of Nile perch to retain its quality.

1.3 Statement of the problem

The Kenyan fishery has no appropriate, simple and rapid method to evaluate the freshness of Nile perch. The industry relies on the EU sensory scheme, which does not account for differences in fish species composition, size and harvesting environment. The scheme groups

several different sensory terms into one quality grade and assumes that the parameters described indicate equal level of deterioration in every fish species. There is therefore a need to develop a method where the descriptions are specific for each fish species, objective and independent of each other to evaluate the quality of the Nile perch. The QIM scheme to be developed will be specific and will group several sensory terms which can be identified with the different quality grades of Nile perch. This will be useful for grading the Nile perch as a species in terms of freshness.

1.4 Objectives

1.4.1 General objective

To develop a Quality Index Method scheme for evaluating the freshness of the Nile perch from L. Victoria and for determining its shelf life in ice.

1.4.2 Specific objectives

a) To select high impact sensory parameters for the development of a Quality Index Method Scheme specific to Nile perch.

b) To determine the trend in the levels of the selected sensory, microbiological and chemical parameters during the storage period of Nile perch in ice.

c) To correlate the sensory, chemical and microbiological parameters.

d) To determine the shelf life of iced Lake Victoria Nile perch using sensory, microbiological and chemical parameters.

1.5 Null hypotheses

a) The selected sensory parameters are not appropriate for developing a Quality Index Scheme for the Nile perch.

b) No trend can be established for the levels of microbiological and chemical parameters during the storage of Nile perch in ice.

c) There is no correlation between the sensory, chemical and microbiological parameters.

d) The selected sensory, chemical and microbiological parameters are not appropriate for determining the shelf life of iced Lake Victoria Nile perch.

1.6 Justification

There is no specific, precise, objective, independent and rapid method for evaluating the freshness of the Lake Victoria Nile perch. The fishing industry relies on the EU sensory scheme, which does not account for the differences between the species of fish and assumes that the characteristics described for the parameters are the same for every fish and have equal rate of deterioration. The fishery also relies on microbiological and biochemical analysis, which cannot be extensively used for rapidly determining the quality of Nile perch as it is landed. The Quality Index Method scheme will be a rapid, precise, objective and independent method for evaluating the freshness of Nile perch, as the method requires no specialized equipment. It is better than relying on laboratory tests which are expensive in terms of requirements for minimum education, technical expertise and resources required and take longer to establish the quality of fish under investigation. The schemes only require that the handler, processor or inspector understands the vocabulary of terms to be used in describing the condition of the fish. The scheme developed will be useful to the fishermen, fish processors and exporters to assess the freshness and accurately predict the shelf life of Nile perch when stored in ice.

1.7 Expected Output

1. A Quality Index Method scheme which will allow quick decisions to be made regarding the quality and freshness of the Nile Perch by fishermen, Fish inspectors, processors and exporters.
2. An accurate prediction of the shelf life of Nile perch from Lake Victoria when stored in ice by the fishermen, fish inspectors and processors.

CHAPTER TWO
LITERATURE REVIEW

2.1 Freshness and spoilage of fish

Freshness is one of the most important aspects of fish and because of consumer preferences, there is a strong tendency to select very fresh fish (Luten and Martinsdottir, 1997). Freshness in fish can be defined as the presence of characteristic appearance, odour, taste and texture. Loss of freshness of seafood is a consequence of postmortem biochemical, physicochemical and microbiological processes which are characteristic of each species, of handling on board and after landing, and of technological processing. These changes are appreciated in sensory terms and can be evaluated by sight, touch, smell and taste (Huidobro *et al.*, 2000). The characteristic sensory changes occur in the appearance, odor, taste and texture of fish during deterioration and thus sensory methods are commonly used for quality assessment by the inspection services and in the fishing industry (Huss, 1995).

Fish spoilage is a complex phenomenon in which different biochemical, microbiological and environmental factors are involved. Bacterial activity is the main cause of spoilage for fish kept above 0°C (Shewan, 1971). The microbiological spoilage of fish is a consequence of microbial growth and activity, which manifests itself as changes in the sensory characteristics (Gram and Huss, 1996). The bacterial degradation of soluble low molecular weight components produces volatile metabolites, such as trimethylamine nitrogen, hydrogen sulphide and ammonia which are responsible for the unpleasant and offensive odours and flavours (Shewan and Murray, 1979). The productions of extracellular polysaccharide materials manifest themselves through slime formation. The growth of moulds, bacteria and yeasts, is manifested as large visible pigmented or non-pigmented colonies on the skin surface during spoilage of fish (Gram and Huss, 1996).

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 (*Pseudomonas* spp., *Shewanella* spp. and *Aeromonas* spp.). The microflora on tropical fish species is very similar to the flora on temperate species, although tropical fish often carry a higher load of gram-positive and enteric bacteria (Gram and Huss, 1996). *Pseudomonas* spp. and enterobacteriaceae (*Salmonella* spp, *Vibrio* spp.,) are shown to dominate the flora at the point of spoilage (Gram *et al.,* 1990).

The non-protein nitrogen fraction (NPN) of the fish consists of low molecular weight water soluble nitrogen containing compounds such as free amino acids and nucleotides and is readily available as bacterial substrate. The decomposition of the sulphur containing amino acids cysteine and methionine is particularly important in spoilage, as it causes off-odours and off-flavours due to the formation of hydrogen sulphide and methylmercaptane, respectively (Herbert and Shewan, 1976).

Fish acts as a substrate for bacterial growth. There are several important specific intrinsic factors in fish, which greatly influence its spoilage. These are the poikilothermic nature of the fish and its aquatic environment, the high post mortem pH in the flesh (>6.0), the presence of large amounts of non-protein nitrogen (NPN) and the presence of trimethylamine oxide (TMAO) as part of the non-protein nitrogen fraction (Herbert *et al.,* 1975).

Deterioration of the quality of fish is characterized by an initial loss of fresh fish flesh flavor (sweet and seaweedy). The initial quality loss in fish is primarily caused by autolytic changes and is unrelated to microbiological activity (Huss, 1995). The degradation of the nucleotides (ATP-related compounds), which is caused by autolytic enzymes, is of particular importance in this respect. The loss of the intermediate nucleotide, inosine monophosphate (IMP) is responsible for the loss of fresh fish flavor (Hanna, 1992). The autolytic changes contribute to spoilage mainly by making catabolites available for bacterial growth (Gram *et al.,* 1990 and Huss, 1995).

2.2 Total volatile basic nitrogen (TVBN)

Total volatile basic nitrogen (TVBN) is one of the most widely used parameters for assessment of seafood quality (Pacheco-Aguillar *et al*., 2000; Papadopoulos *et al*., 2003; Rodriguez *et al*., 1999) In general terms, it includes the measurement of trimethylamine (TMA) produced by spoilage bacteria such as *Pseudomonas* spp. and *Shewanella 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.*, 2008). TVBN was found not to change very much during the first ten days of storage of Lake Victoria Nile perch in ice (Gram *et al.,* 1989). In fresh Nile perch TVBN contents varied between 9 and 11 mgN/100g. In the spoilt Lake

Victoria Nile perch that was kept in ice after a delay of 3-6 hours, TVBN reached 28 mgN/100g after 20 days in ice (Karungi *et al*., 2004),

2.3 Trimethylamine oxide (TMAO) and trimethylamine nitrogen (TMAN)

Trimethylamine oxide is an osmoregulatory compound in marine teleosts. It is one of the components of non protein nitrogen (NPN). It is used as an index in assessing the freshness and shelf life of fish. The spoilage of fish is influenced by trimethylamine oxide (TMAO), particularly under conditions where oxygen is excluded. Trimethylamine oxide is known to cause a high (positive) redox potential (E_h) in fish flesh (Huss and Larsen, 1980). A number of well defined spoilage bacteria (*Shewanella putrefaciens, Photobacterium phosphoreum, Vibrionaceae)* are able to utilize trimethylamine oxide as the terminal electron acceptor in anaerobic respiration resulting in off-odours and off-flavours due to the formation of trimethylamine nitrogen (TMAN) (Gram *et al.*, 1990).

In fresh fish in the tropics, the reduction of TMAO yields TMAN by reductase positive microorganisms like *Psuedomonas spp. Shewanella putrefaciens, photobacterium phosphoreum, Vibrionaceae* and therefore results in rapid accumulation of TMAN in the fish muscle under refrigerated condition This is accompanied by a corresponding decrease in TMAO content (El Marrackchi *et al,* 1990).

Trimethylamine oxide was found to be 11 mgN/100g in fresh Nile perch weighing 0.5 to 1 kilograms compared to 25-35 mgN/100g for fresh Nile perch weighing more than 3 kg. TMAO values varied during storage between 13 and 35 mgN/100g and tended to decrease in some samples as storage progressed (Gram *et al.,* 1989). In cod and other gadoid fishes, TMA constitutes most of the TVBN until spoilage. However, in the spoiled fish where TMA has reached its maximum level, TVBN levels still rise due to the formation of ammonia (Huss, 1995). In some fish where spoilage is due to a non-TMAO reducing flora, a slow rise in TVBN is detected during storage, probably resulting from the deamination of amino acids (Dalgaard *et al.,* 1993).

2.4 pH

The high post mortem pH (>6.0) and very little carbohydrate (<0.5%) in the muscle tissue with small amounts of lactic acid produced at post-mortem, have important consequences on the microbiology of fish. The high pH allows the sensitive spoilage bacterium *Shewanella*

putrefaciens to grow (El Marrackchi *et al,* 1990). Post mortem gycolysis results in the accumulation of lactic acid, which in turn lowers the pH. This has an effect on the physical properties of the muscle. As the pH drops, the net surface charge on the muscle proteins is reduced, causing them to partially denature and lose some of their water holding capacity. Loss of water has an effect on the texture leading to softening of the muscle (Huss, 1995).

2.5 Lipid hydrolysis

Post-mortem hydrolysis of fish muscle lipids lead to the release of free fatty acids. During storage, a considerable amount of free fatty acids (FFA) appear. This phenomenon is more profound in ungutted than gutted fish and is catalyzed by lipases and phospholipases (Pacheco-Aguilar *et al.,* 2000). In ungutted fish stored in ice, lipases from the internal organs get released into fish muscle where lipids are localized and therefore contribute increasingly to lipolytic breakdown of fish lipids (Chaijan *et al.*, 2006). Triglycerides in the depot fat are cleaved by triglyceride lipase originating from the digestive tract or excreted by certain micro-organisms such as *Psuedomonas fragi*. Cellular lipases like phospholipase A_2 may also play a minor role. The fatty acids bound to phospholipids at glycerol-carbon atom 2 are largely of the polyunsaturated type and hydrolysis therefore often leads to increased oxidation as well (Nayak *et al.*, 2003). The fatty acids may cause a soapy off-flavor (Huss, 1995).

2.6 Sensory evaluation

Generally, consumer acceptance of food products is determined by sensory quality. It is therefore extremely useful to have methods for describing the sensory properties of foods as a means of ascertaining their initial sensory characteristics and any changes undergone by the product in the course of storage (Huidobro *et al.*, 2000). Sensory evaluation therefore is an important method for the assessment of freshness and quality and is commonly used in the fish sector and fish inspection services (Luten and Martinsdottir, 1997).

Sensory analysis has the advantage of being rapid and simple. However, it has a certain degree of subjectivity, which is only partially avoided by using an expert and extensively trained panel. Over the last 50 years, a large number of sensory schemes have been developed for sensory analysis of raw fish (Baixus-Nogueras *et al.*, 2003). In Europe, the most commonly accepted method is the EU scheme, introduced in 1976 and updated in 1996 (EEC, 1996). This method is widely used for a variety of fish and has been satisfactorily correlated with chemical

parameters, such as volatile amines (Perez-Villareal and Howgate, 1987), microbial counts (Pastoriza *et al.*, 1998) and time of ice storage (Koutsouminas and Nychas, 1999). However, its suitability has been questioned because, in using general parameters, it does not take into account particular differences among species (Baixus-Nogueras *et al.*, 2003). This has led to the development of an alternative and more specific sensory method known as the Quality Index Method (QIM) (Bremner, 1985).

2.7 The Quality Index method

The Quality Index Method (QIM) is a sensory method that is based on detailed descriptors that are grouped into distinct characters within general attributes such as general appearance, eyes and gills (Luten and Martinsdottir, 1997). It is a descriptive, fast and simple method to determine freshness quality (Bremner, 1985; Branch and Vail, 1985; Bremner *et al.*, 1987). The method is useful essentially because it evaluates those sensory parameters and attributes that change most significantly in each species during degradation processes (Hyldig and Nielsen, 1997).

The Quality Index Method is based on assigning a range of demerit points to a set of characteristic attributes of each parameter that is in direct proportion to their importance in the deterioration pattern of the species. The sum of the points awarded to each parameter gives a total score, which is the Quality Index at the time of assessment (Luten and Martinsdottir, 1997; Hyldig and Nielsen, 1997). As the various parameters are considered, the contribution of each one to the total is a relative value, and changes in attributes do not markedly unbalance the final assessment. In this way a linear relationship can be established between the freshness quality expressed by the index and the storage time in ice. The maximum Quality Index score is therefore reached at the point in storage when the sensory panel rejects the cooked product (Luten and Martinsdottir, 1997; Sveinsdottir *et al.*, 2003).

A Quality Index (QI) score when applied to raw fish can be used to predict the remaining storage time in ice. This provides a new alternative to the methods based on structured scales, which had been used for raw fish (Howgate *et al.*, 1992; Botta, 1995). This method has been recognized as a fast and reliable approach to assess the freshness of fish (Botta, 1995). The usefulness of this tool is further improved when new schemes applicable for particular fish species or products are developed (Dalgaard, 2000). The Quality Index Method schemes have currently been developed for a number of fish species including: fresh herring (*Clupea harengus*)

and cod (*Gadus morhua*) (Larsen *et al.*, 1991; Jonsdottir, 1992;), Atlantic mackerel (*Scomber scombrus*), horse mackerel (*Trachurus trachurus*), European sardine (*Sardina pilchardus*) (Andrade *et al.*, 1997), brill (*Rhombus laevis*), dab (*Limanda limanda*), haddock (*Melanogrammus aeglefinus*), Pollock (*Pollachius virens*), sole (*Solea vulgaris*), turbot (*Scophthalmus maximus*) (Luten, 2000; Matinsdottir *et al.,* 2001), gilthead seabream (*Sparus aurata*) (Huidobro *et al.*, 2000) and farmed Atlantic salmon (*Salmo salar*) (Sveindottir *et al.*, 2003). The author is aware that no QIM scheme has been developed for Nile perch as for many other tropical fish. The development of the QIM scheme for a particular seafood or fish species involves the selection of appropriate and best fitting attributes in order to observe a linear increase in the Quality Index with storage time in ice (Dalgaard, 2000).

2.8 Shelf life of fish

The activity of micro-organisms is the main factor limiting the shelf life of whole fresh fish. An estimation of the total viable counts (TVC) is used as an acceptability index in standards, guidelines and specifications (Olafsdottir *et al.,* 1997). Newly caught fish contain a diverse microflora. Total viable counts of 10^2 to 10^6 cfu/g are usual on whole fish and cut fillets. During chill storage, psychrotolerant micro-organisms are selected, thus differential counting of these micro-organisms is suggested as a measure of fish quality (Gram *et al.,* 1990). At the point of sensory rejection, the TVC of fish are in the range of 10^8- 10^9 cfu/g. At this point the sensory panel or part of the panel detects spoilage attributes in the samples or when the samples are increasingly described with words applying to spoilage attributes such as fishy taste, sour/stale sour or softer/gritty texture (Stone and Sidel, 1998).

Fish guts are known to be a reservoir of digestive enzymes and bacteria. The digestive enzymes cause autolysis at post mortem which gives rise to strong off-flavors and off-odours especially in the belly area and at times causes belly burst (Tejada and Huidobro, 2002). The gutting process therefore destroys the reservoir of these digestive enzymes and bacteria thereby reducing the process of autolysis in fish. Gutting has been reported to have an effect in extending the shelf life of fish species during chill storage (Rodriguez *et al.*, 1999). The process of gutting has also been reported to delay the ripening process in anchovies under chilled storage conditions (Mendes *et al*., 1999). Regarding the frozen storage of fish, gutting has been shown to maintain sensory qualities and reduce volatile amine contents in lean fish (Botta *et al*., 1982) as compared

to ungutted fish. Nevertheless, gutting did not afford any significant effect on quality when frozen fatty fish was considered (Karacam and Boran, 1996).

Quantitative Descriptive Analysis (QDA) is a method used to determine the shelf life of fish by the sensory evaluation of cooked fish samples. In this method, an unstructured scale of 0 to 10 is used on a list of words describing odour, flavor and texture (Sveinsdottrir *et al.*, 2003). QDA serves well for the identification of specific sensory characteristics of products pertinent to consumer preferences (Sidel *et al.*, 1994; Helgesen *et al.,* 1997 and Reyes-Vega *et al.*, 1998). The method has been used to relate sensory textural attributes to instrumental measurements and in determining the shelf life of products without dependence on standards or control products. When establishing the shelf life of a product, the limit of consumption may be determined, when the panel or part of the panel detects spoilage characteristics in the samples. As pointed out by Stone and Sidel (1998) accurate information on which quality characteristics are considered to be the most important to consumers is a valuable tool for quality control in food processing. The results from QDA may be used as a reference when developing QIM schemes for fresh fish.

2.9 Effect of ice on fish storage

Utilization of ice to preserve fish dates back to more than a century. Icing reduces the temperature of fish to about 0°C which therefore reduces the growth of spoilage and pathogenic micro-organisms hence a decrease in the rate of spoilage. The reduction in temperature also reduces the rate of enzymatic reactions in particular those linked to early post mortem changes (Ward, 1994). The reduction of temperature due to icing may cause a number of physiological changes by changing metabolic pathways and end products of bacteria. *Pseudomonas* spp. are known to produce lipase and protease at low temperatures which catalyses the rate of lipid deterioration and fish muscle components breakdown, respectively.

Ice is more appropriate in preserving fish because it has a large cooling capacity. The latent heat of fusion of ice is about 80 kcal/kg, which means that a small amount of ice would be needed to cool 1 kg of fish. Ice has a number of practical properties that makes its use advantageous. It is a portable cooling method and therefore can be easily stored, transported and used. Ice is a safe food grade substance if produced from potable water. Ice can be produced in different shapes and the most commonly utilized in fish storage are flake, plate, tube and block ice. The block ice is usually ground before being utilized to chill fish. In general, flake ice

normally allows for an easier, more uniform and gentle distribution of ice around fish and produces very little or no mechanical damage (Huss, 1995).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study site

The study site was the fishing grounds under the jurisdiction of Kisumu District in the Nyanza gulf of Lake Victoria. The fish samples were purchased at Ogal and Dunga beaches.

3.2 Sampling

3.2.1 Sampling of fish

Fresh Nile perch (*Lates niloticus*) were caught and immediately iced after being taken off the nets at the fishing ground (control samples). The fish samples were divided into two groups with one group ungutted and the other gutted. Another set of fish samples were bought from the fishermen at the beaches (experimental samples) after approximately 3-4 hours of delayed icing from the time they were caught and were also divided into two groups (gutted and ungutted) and iced. The weights and lengths of the whole fish were taken and the fish were immediately layered with flaked ice and packed in insulated containers.

The gutting process was done at the landing beaches. The fish were washed with running drinking tap water to remove any dirt or slime. The fish were placed on clean stainless steel surface and inverted with the belly facing upwards. The anal pores of the fish were located and cut in a V or notch shape. The knife was pointed into the cut and drawn towards the head splitting the fish to the base of the gills. The fingers of the hand holding the knife were used to remove the gut from the cavity. The fish guts were discarded in a collection plastic bin. The fish were finally washed in drinking tap water and layer iced immediately.

A set of thirty fish was purchased each sampling time. A total of 360 fish were used in this experiment. The sizes of the fish sampled were between 45 and 50 cm in length which were within the statutory specifications (Fisheries Department, 1991). The iced Nile perch were transported to a nearby chill store and stored at 0-2°C overnight before being transported the next day to the Food Chemistry Laboratory at Egerton University 150 km away within 4-5 hours, where they were kept under similar conditions. Three fish were randomly sampled on days 2, 6, 10, 14, 18, 22, 26 and 30 of storage in ice. During the storage period, any melted ice was replaced. All experimental samples were analyzed using the sensory, biochemical and microbiological tests.

3.2.2 Water sampling

Water samples were taken from the fishing ground, shore, boats and taps. The water samples were collected in 300 mL clean sterile bottles. The bottles were held at the bottom by right hand and the caps removed by left hand. Care was taken to protect the sterile cap and bottle neck from contamination by keeping the bottles away from the other body parts. For the fishing ground, shore and boat water, the bottles were plunged with the neck downwards below the water surface. The bottles were then filled with water by a sweeping forward motion and bringing the bottle up towards the surface. The bottles were then recapped and kept in ice. The samples were transported to the laboratory at Egerton University and analysed within six hours.

For the tap water, the tap was sterilized by flaming the threads of the tap with a lighter for one minute. The water was left to run freely for at least five minutes and then the faucet was partially closed to avoid splashing. The sampling bottles were held near the bottom with right hand and the cap unscrewed with the left hand taking care not to touch the part of the cap that touches the neck of the bottle. The sampling bottles were held one inch below the stream of tap water and filled to the neck without allowing the sample bottle to overflow. The sampling bottles were then removed from under the flow and immediately recapped tightly. The samples were kept in ice and transported to the laboratory and analysed within six hours.

3.3 Research design

A complete randomized block design was used in the experiment with the treatments being the different days of storage in ice and blocks being the fish samples from the fishing ground (gutted and ungutted) and beaches (gutted and ungutted). Eight treatments were randomly assigned to the experimental units. Each batch of three fish samples was subjected to the same storage duration and was considered to be an experimental unit. The control consisted of fish samples that were caught from the fishing ground.

3.4 Laboratory Analyses

3.4.1 Sensory Analysis

3.4.1.1 Preliminary studies on developing the proposed QIM scheme for raw Nile perch

To develop the QIM scheme for Nile perch, preliminary sensory studies were carried out by a panel of three experts to establish the most significant sensory characteristics associated with Nile perch spoilage during storage in ice. The selected parameters were: the appearance of the

skin and slime; the clarity and shape of the eyes; flesh color in open surfaces; the extent of adherence of the peritoneum; flesh elasticity and belly texture; and gill color, odor and slime. The criteria for the development of the preliminary quality index scheme was based on the sensory characteristics which are used by the fishermen, fish traders and fish inspectors in evaluating the freshness of fish. The guidelines for sensory evaluation for fish species as proposed by Martinsdottir *et al*. (2001) was also used in developing the preliminary sensory scheme.

Panels consisting of 10 judges were trained and used in sensory analysis. The judges were all employees and students of the Department of Dairy, Food Science and Technology of Egerton University who have experience in assessing fish for consumer acceptability. The training was performed to familiarize them with the sensory scheme being developed for Nile perch freshness evaluation. Eight training sessions were organized to get uniformity of sensory evaluation. The descriptive testing method with structured scaling was used to determine the nature and intensity of different parameters tested.

Training of panelists was based on the preliminary scheme formulated by the three experts. The scheme was explained to the panel while observing the fish of different degrees of freshness. This session lasted about 30 minutes every time it was conducted. Different batches with known time of storage and temperature histories were observed and discussed at the end of each session. All suggestions for improvement by the panelist were noted, evaluated and included in the final scheme. In the second stage of training, each Nile perch sample with an unknown storage and temperature history to the panelists was coded with three random digit numbers and presented to the panelists for evaluation. The history of the samples was then made known to the panelists at the end of the sessions. The first ten results of training session that were within 5% of each other were then used as criteria for selecting the panelists.

When it was found that on day 30 the fish had spoiled, the investigator decided to sample a few days earlier, i.e., on day 28 for greater accuracy in determining the end of shelf life on ice. The changes observed on the above parameters during ice storage were listed in a preliminary QI scheme. However, the expert parameter selection panel found out that the changes in the flesh color in open surfaces and the adherence of the peritoneum were more difficult to discern and therefore left them out in the final list of parameters used in the experiments that followed. The final version of the QIM scheme was finalized and presented for discussion and endorsement by

the panelists. This scheme was then used to assess the freshness of Nile perch stored in ice and is described in this study.

3.4.1.2 Sensory evaluation of cooked Nile perch fillets

This was done to determine the perception of the panelists on the different organoleptic properties that can used to determine the shelf life of Nile perch. The attributes of cooked fish (odor, taste and texture) were evaluated by the same panel on each sampling day simultaneously as the raw fish was evaluated. The cooked fillet samples were taken from the same region of the same fish that was used for the raw fish evaluation. The sensory assessment score sheet for cooked fish was adopted from Huss (1995) (Appendix 1). The fish samples (50 g) were cooked at 95°C for 25 minutes, cooled and immediately presented to the panelists. The odor, taste and texture were evaluated using a structured acceptability scale of 0-10. The rejection threshold was set at Ò4 for each of these three attributes.

3.4.2 Biochemical Analyses

3.4.2.1 pH

This was done to establish the negative hydrogen ion concentration in the fish as an intrinsic factor in affecting the growth and survival of micro-organisms during storage of Nile perch in ice. This was carried out according to procedures described by Goulas and Kontominas (2005).Ten grams of Nile perch fillet from the tail region were homogenized in 50 mL of distilled water and the mixture filtered using Whatman filter paper No.1. The pH of the filtrate was measured using a CRISON Model 507 pH meter ((Barcelona, Spain) at ambient temperature after calibration of the instrument using standard buffers of pH 7 and 4 at 25°C. The pH meter could read measurements accurately to ±0.01 pH units.

3.4.2.2 Total volatile basic nitrogen (TVBN)

This was carried out to determine the extent of bacterial breakdown of protein component of the fish flesh into volatile nitrogenous amines which may be responsible for the deterioration in the quality of fish flesh. Approximately a-10 gram sample of skinless fish flesh from the tail region was homogenized with 50 mL of distilled water. The mixture was centrifuged in Sorval RC-285 centrifuge (Dupont, Wilmington, Delaware, U.S.A) at 400 rpm for 5 minutes and the supernatant filtered through a Büchner funnel using Whatman No.1 filter paper. Two grams of

MgO was added followed by one drop of silicon antifoaming agent. A 250-mL Erlenmeyer flask containing 25 mL of 3% aqueous solution of boric acid, 0.04 mL of a mixture of methyl red and methylene blue indicators was used as the indicator. Distillation was continued until a final distillate volume of 125 mL was obtained. The distilled TVBN was titrated with an aqueous 0.1N HCL solution. TVBN content was expressed as mgN/100g of fish flesh.

TVBN = (V × C × 14 × 100) /10, where V is the volume of hydrochloric acid added and its concentration (C), 10 represent the weight of the sample while 14 is the molecular weight of nitrogen (Goulas and Kontominas, 2005).

3.4.2.3 Determination of trimethylamine nitrogen and trimethylamine oxide

This was done to establish the presence of TMAO which is an osmoregulatory compound in some fish species and the extent to which the component was reduced to trimethylamine nitrogen through bacterial action. Trimethylamine nitrogen (TMAN) was determined using the TVBN method described above in 3.4.2.2 as modified by Malle and Tao (1987). Formaldehyde was used to block the primary and secondary amines. A-100 g sample of skinless fish flesh was mixed with 100 mL of distilled water. The mixture was quantitatively transferred with 30 mL of distilled water into a round-bottom flask and was distilled after the addition of 2 g of MgO, 100 mL of 20% aqueous solution of formaldehyde (HCHO) and five drops of silicon antifoaming agent. The same procedure was used as for TVBN determination (section 3.4.2.2) and 125 mL of distillate was collected. The distillate was titrated using aqueous 0.05 N hydrochloric acid solution. The amount of TMAN in mg/100g of fish flesh was calculated from the volume (V) of hydrochloric acid added and its concentration (C) as follows: % mg TMAN = (V×C×14) (Goulas and Kontominas, 2005). Trimethylamine oxide was determined after reduction with titanium (III) chloride ($Ticl_3$) and then calculated after subtracting trimethylamine content of the samples (Benjakul *et al*., 2004).

3.4.2.4 Free Fatty Acids (FFA) determination.

This was done to monitor the extent of hydrolysis of fish lipids during the period of storage of Nile perch in ice. Percent free fatty acids were determined by the standard AACC Method (2004). Seven grams of the *Lates niloticus* was accurately weighed in duplicate and 100 mL of hot neutral alcohol added. Titration was done with 0.1N NaOH after addition of Phenolphthalein indicator. % FFA= (mL×N×F×100/sample wt) ×1000. Where mL= mL of NaOH required, N=

normality of NaOH solution, F=equivalent weight of FFA in which results are to be expressed. FFA is usually expressed as % oleic acid and equivalent weight is 282/1 or 282.

3.4.3 Microbiological Analyses

3.4.3.1 Total Viable Count (TVC)

The TVC was carried out to determine the initial gross microbial contamination of fish samples at harvest and during handling. About 25 cm^2 of the skin of fish samples were rinsed with 70% ethanol and sample was taken from the flesh of the anterior dorsal region of each of three whole fish. The skin was aseptically removed and 25 g of the underlying flesh were sampled using sterile scalpels and forceps and were mixed with 225 ml of buffered peptone water diluents and homogenized. Six fold serial dilutions were made using buffered peptone water. Sterile duplicate petridishes were labeled according to the dilution index. The total viable counts (TVC) were cultured on Iron Agar (Oxoid, UK) by pour plate technique with an overlay as described by Gram *et al.* (1987). The plates were incubated at 22°C for 72 hours for psychrophilic bacteria and at 37°C for 24 hours for mesophilic bacteria. The TVC was obtained by registering all bacterial colonies on the incubated plates from each dilution of the sample showing growth and able to be counted.

3.4.3.2 Hydrogen sulphide producing bacteria

The H_2S producing bacteria counts were determined to assess the presence of bacteria which were able to produce H_2S on degradation of proteins that may be responsible for off-flavours and off-odours in the fish samples. About 25 cm^2 of the skin of fish samples were rinsed with 70% ethanol and sample was taken from the flesh of the anterior dorsal region of each of three whole fish. The skin was aseptically removed and 25 g of the underlying flesh were sampled using sterile scalpels and forceps and were mixed with 225 ml of buffered peptone water diluents and homogenized. Six fold serial dilutions were made using buffered peptone water. Sterile duplicate petridishes were labeled according to the dilution index. The hydrogen sulphide producing bacteria were cultured on Iron Agar (Oxoid, UK) by pour plate technique with an overlay as described by Gram *et al.* (1987). The plates were incubated at 22°C for 72 hours. The number of colonies showing a clear dark color represented the bacterial population able to produce H_2S.

3.4.3.2 Coliforms

This was determined to assess the conditions that might introduce hazardous organisms or allow proliferation of enteric pathogenic organisms. The analysis was carried out according to the FDA (1998) procedure. The drip fluids and water on the surface of the *Lates niloticus* were used to assay for coliforms. Six fold serial dilutions were prepared. The diluents were cultured onto Violet Red Bile Lactose Agar (VRBLA) by pour plating and incubated at 37°C for 48 hours. After 48 hours, colonies were enumerated and results reported as colony forming units per gram (c.f.u g^{-1}). Purple red colonies surrounded by a whitish zone of inhibition by bile acids were counted. To confirm whether they were coliforms at least ten representative colonies were picked and transferred to ten tube of Brilliant green lactose bile broth. Gas and acid production was examined after 24 to 48 hours (FDA, 1998).

3.4.3.3 *Escherichia coli*

This was done to assess the indication of contamination of relatively recent fecal origin. Suspected colonies from VRBLA were isolated and identified. The colonies were streaked on Levinesô Eosin-Methylene Blue Agar (L-EMB) and incubation done at 35°C for 18 to 24 hours. Characteristic colonies appearing with greenish metallic sheen were identified in L-EMB agar. Indole, Methyl red, Voges-proskauer and Citrate (IMVIC) test was then carried out on the suspected colonies by inoculation (FDA, 1998).

Indole test: Pure colonies of isolates were suspended in peptone water medium and incubated at 37°C for 24 hours. One mL of Kovacôs reagent was added and shaken gently. It was left to stand for 5 minutes. A pink to red colour development was positive for indole production.

Methyl red test: Pure colonies were inoculated in MR-VP medium tubes and incubated at 37°C for 24 hours. Two drops of methyl red solution was then added and shaken gently. Red colour was positive while yellow colour was negative.

Voges-proskauer test: After completion of the MR test 0.6 mL of 5% alpha naphthol solution and 0.2 mL of 40% potassium hydroxide was added and shaken. The tubes with the mixture were then sloped and incubated at 37°C and examined after every 15 minutes up to 4 hours. Pink to brisk red colour showed a positive reaction.

Citrate test: Pure colonies were inoculated in a tube of Koserôcitrate medium. Incubation was done at 37°C and examination done after 4 days for growth.

Escherichia coli were Indole positive, Methyl red negative, Voges-proskauer negative and Citrate negative.

3.4.3.4 *Salmonella* spp

Twenty five grams of skinless *Lates niloticus* raw flesh were weighed and aseptically added to 225 ml of buffered peptone water and homogenized with a blender. The mixture was incubated at 37°C for 24 hours. After the incubation, the mixture was shaken gently to mix well, then using a sterile pipette, 1 mL was transferred into 10 mL of Rappaport vassiliadis medium (Difco). This was incubated in a water bath 42°C - 43°C for 24 hours. After incubation, a loopful of the Rappaport Vassiliadis broth (Difco) culture was streaked on Xylose lysine desoxycolate (XLD) agar (Oxoid) and brilliant green agar BGA (Himedia, India) and incubated at 37°±1°C for 24 hours. Colonies that appeared dark on XLD and those that appeared pink in BGA were taken to be non lactose fermenters and were purified on MacConkey agar (Himedia, India). The colonies that appeared shiny-yellow mucoid on both BGA and XLD were lactose fermenters and were also purified on MacConkey agar. The purified non lactose fermentersô colonies on MacConkey agar were inoculated into triple sugar iron (TSI) agar slants by stubbing the butt and streaking the slant. Incubation was then done at 37°C for 24 hours. No colour change was observed on the slant and the butt (FDA, 1998).

3.4.3.5 Coliforms in water

This was determined to indicate the quality level of the water in which the fish was caught and hygienic handling at post harvest step. The analysis was carried out according to the FDA (1998) procedure. Inoculation was done on 5 tubes of lauryl tryptose broth of 10 mL quantities (double strength) each with 10 mL quantities of water sample, five tubes of the medium (single strength) of 5 mL quantities each with 1 mL water sample and another set of 5 tubes of 5 mL of the medium (single strength) with 0.1 mL of the water sample. These were incubated at 35°C for 24 to 48 hours. Gas presence or absence was recorded. Tubes showing gas formation were gently shaken and 1-3 loopfuls of the medium was transferred to fermentation tubes containing brilliant green lactose bile (BGLB) broth and further incubated for 24 hours at 35°C. A loopful from each positive BGLB broth tube was transferred and streaked in two L-EMB plates and incubated at 35°C for 24 hours. Pure and well isolated pinkish colonies were transferred to lauryl tryptose broth and to a nutrient agar slant and incubated at 35°C and gas formation recorded. Gas

formation in fermentation tubes was then recorded and a gram stained preparation from each of the agar slant cultures made.

3.4.4 Proximate analysis

3.4.4.1 Moisture content determination

Moisture content was determined as an extrinsic factor affecting growth of microorganisms by oven drying of 5 g of fish flesh in triplicate at 105°C until a constant weight was reached (AOAC, 2000).

3.4.4.2 Crude protein determination

This was done to determine the level of protein content over the storage period of Nile perch in ice. The AOAC (2000) procedure was used. Approximately 0.2 grams of *Lates niloticus* flesh was accurately weighed in triplicate into three Kjeldhal digestion tubes. Ten mL of concentrated sulphuric acid was added into each of the tubes and one selenium tablet added to act as a catalyst. The samples were then digested at a temperature of 445°C for 3 hours. The samples were cooled to room temperature and then distilled using Kjeldhal distillation unit (UDK 127, Velp Scientifica, Spain). The distillate was collected in 15 mL of 0.1N HCL in which a mixed indicator of methyl red and methylene blue had been added. The HCL was then titrated against 0.1N NaOH. The % crude protein was determined by the following formula:

% Crude protein = {(V1- V2) × N × 14 × 6.25/W}, where V2 is the volume of the titre, V1 is the volume of HCL used for the blank test, N is the normality of acid and W is the weight of the test portion.

3.4.4.3 Crude Lipids determination

This was done to establish the content of lipid during the storage period of Nile perch in ice. The AOAC (2000) procedure was used. Five grams of the *Lates niloticus* flesh was accurately weighed into Soxhlet thimble in triplicate. The sample was covered with cotton wool and the thimble placed in the Soxhlet extractor (CX7/06, Quickfit, England). The extractor was connected to a flat-bottomed flask in which 200 mL of hexane had been added and the mixture heated on a heating mantle. The sample was then refluxed for 6 to 8 hours and the solvent evaporated in a rotary evaporator (RE52CS-2, Shanghai, China). The sample was oven dried at 80°C for 30 minutes (to constant weight) and cooled in a desiccator and then weighed.

The lipid content was determined by the following formula:
% crude lipid = (wt of residue/original wt of sample) x 100.

3.4.4.4 Ash estimation

This was determined according to AOAC (2000). A clean labeled, flat-bottomed silica dish was heated to bright redness over a Bunsen flame then allowed to cool in a dessicator before weighing (wt.x). Approximately 3 grams of the fish sample was weighed in triplicate and added to the dish and then weighed accurately (wt.y). The dish was finally placed in the muffle furnace at 520°C and heated until the ash was greyish. The dish was carefully removed from the furnace and placed on the dessicator to cool. The cooled dish was then weighed and the weight recorded (wt.z). The Ash content was determined by the following formula % = $\frac{(\text{wt z ï wt x})}{(\text{wt y ï wt x})} \times 100$.

3.5 Data Analysis

The data from the three sampling times were pooled and the mean values for the sensory, microbiological and chemical parameters analyzed were determined for each of the treatments. The means of the selected sensory, microbiological and chemical parameters analyzed for each of the treatments (at each sampling period) were subjected to analysis of variance. The least significant difference (LSD) procedure of Fisher was used to test for the difference between the treatments means (significance was defined as pÒ 0.05). The correlations between the different parameters in the study were calculated using SPSS version 15 (SPSS Inc., Chicago, Illinois).

CHAPTER FOUR

RESULTS

4.1 Sensory Analysis

4.1.1 Quality Index Method Scheme

Based on the local fishermen and fish inspectorsô knowledge and the guidelines as provided by Martinsdottir *et al.* (2001), the following parameters were identified for use in the development of preliminary QIM scheme for Nile perch (Table 4.1).

Table 4.1: The selected sensory parameters used in preliminary QIM scheme development for the Lake Victoria Nile Perch (*Lates niloticus*)

Parameters		Attributes	Demerit points
Appearance	Skin	Very bright, pearl shiny	0
		Bright	1
		Dull	2
		Very dull	3
	Slime	Clear- transparent	0
		Slightly cloudy/milky	1
		Cloudy	2
Flesh	Colour (open surfaces)	Bright	0
		Dull	1
	Peritonium	Adhered	0
		Partially separated	1
		Totally separated	2
Eyes	Clarity	Clear-translucent	0
		Slightly opaque	1
		Opaque	2
	Shape	Convex	0
		Flat	1
		Concave/sunken	2
Texture	Elasticity	Elastic (finger mark disappears immediately)	0
		Slightly marked by pressure	1
		Clearly marked by pressure	2
	Belly	Intact	0
		Slightly intact	1
		Soft	2
		Very soft	3
Gills	Colour	Bright/dark red	0
		Brownish red	1
		Discoloured/brown	2
	Odour	Fresh, seaweedy	0
		Neutral	1
		Fishy/sour	2
		Off-odour/rotten	3
	Slime	Clear-translucent	0
		Slightly cloudy	1
		Cloudy	2
		Total demerit points (QI scores)	**24**

After removal of the flesh colour and peritoneum parameters that were considered inappropriate for determining the freshness of ice stored Nile perch, the demerit points reduced to 20. This was used in final QIM scheme development for Nile perch (Table 4.2).

Table 4.2: The final QIM scheme developed for the L.Victoria Nile perch (*Lates niloticus)* stored in ice

Parameters		Attributes	Demerit points
Appearance	Skin	Very bright, pearl shiny	0
		Bright	1
		Dull	2
	Slime	Clear- transparent	0
		Slightly cloudy/milky	1
		Cloudy	2
Eyes	Clarity	Clear-translucent	0
		Slightly opaque	1
		Opaque	2
	Shape	Convex	0
		Flat	1
		Concave/sunken	2
Texture	Elasticity	Elastic (finger mark disappears immediately)	0
		Slightly marked by pressure	1
		Clearly marked by pressure	2
	Belly	Intact	0
		Slightly intact	1
		Soft	2
		Very soft	3
Gills	Colour	Bright/dark red	0
		Brownish red	1
		Discoloured/brown	2
	Odour	Fresh, seaweedy	0
		Neutral	1
		Fishy/sour	2
		Off-odour/rotten	3
	Slime	Clear-translucent	0
		Slightly cloudy	1
		Cloudy	2
		Total demerit points (QI scores)	**20**

n=9

The average weight and length for the Nile perch samples used for this study was 887.2±2.9 grams and 48.6±0.4 cm, respectively. The average temperature at each sampling time was 1.4±0.14°C. Increase in the QI scores with storage days in ice was observed for gutted and ungutted experimental Nile perch. Significant increase in the QI scores was observed on the 10th and 14th day (pÒ0.05) day of storage in ice for ungutted and gutted experimental fish samples, respectively (Table 4.3).

Table 4.3: QI scores for parameters of iced gutted and ungutted experimental Lake Victoria Nile perch (*Lates niloticus)*

Parameters		Days of sampling						
		2	6	10	14	18	22	26
Skin	G.	0.45±0.13^a	0.60±0.13^a	0.75±011^a	0.96±0.04^b	1.1±0.11^b	1.47±0.23^b	1.87±0.12^c
	U.	0.43±0.15^a	0.77±0.15^a	0.83±011^a	0.97±0.05^a	1.1±0.1^b	1.53±0.57^b	N/a
Slime	G.	0.43±0.10^a	0.61±0.14^a	0.71±0.15^a	1.17±0.15^b	1.41±0.10^b	1.63±0.11^b	1.92±0.12^c
	U	0.33±0.10^a	0.70±0.17^a	1.07±0.15^b	1.47±0.12^b	1.83±0.15^b	1.93±0.12^c	N/a
Eyes clarity	G.	0.33±0.01^a	0.37±0.12^a	0.73±0.10^a	1.03±0.12^b	1.37±0.15^b	1.53±0.12^b	1.91±0.15^c
	U	0.40±0.00^a	0.73±0.23^a	1.0±0.00^b	1.23±0.15^b	1.67±0.21^b	1.93±0.12^c	N/a
Eyes shape	G.	0.27±0.01^a	0.30±0.15^a	0.81±0.12^a	1.10±0.05^a	1.40±0.10^b	1.61±0.14^b	1.90±0.10^c
	U	0.20±0.02^a	0.37±0.20^a	1.03±0.15^b	0.93±0.02^a	1.7±0.17^b	1.9±0.10^b	N/a
Elasticity	G.	0.25±0.11^a	0.33±0.11^a	0.78±0.16^a	1.20±0.10^b	1.50±0.11^b	1.73±0.02^b	1.93±0.02^b
	U	0.27±0.15^a	0.47±0.23^a	0.9±0.26^b	1.47±0.05^b	1.67±0.21^b	1.97±0.02^c	N/a
Belly	G.	0.30±0.21^a	0.55±0.15^a	0.73±0.18^a	1.25±0.12^b	1.91±0.22^b	2.31±0.21^c	2.70±0.07^c
	U	0.37±0.23^a	0.83±0.25^a	1.23±0.25^b	1.5±0.10^b	2.2±0.26^c	2.57±0.41^c	N/a
Gill color	G.	0.37±0.13^a	0.55±0.25^a	0.81±0.13^a	1.04±0.13^a	1.32±0.15^b	1.57±0.12^b	1.90±0.12^c
	U	0.43±0.23^a	0.83±0.4^a	0.93±0.23^b	1.26±0.23^b	1.4±0.26^b	1.70±0.10^b	N/a
Gill odor	G.	0.30±0.15^a	0.43±0.12^a	0.70±0.10^a	1.41±0.15^b	1.53±0.21^b	2.25±0.25^c	2.71±0.08^c
	U	0.33±0.15^a	0.63±0.11^a	1.00±0.10^b	1.60±0.20^b	2.0±0.65^c	2.57±0.45^c	N/a
Gill slime	G.	0.45±0.15^a	0.63±0.15^a	0.89±0.03^a	1.53±0.12^b	1.48±0.11^b	1.63±0.12^b	1.94±0.08^c
	U	0.43±0.15^a	0.83±0.15^a	0.97±0.02^b	1.33±0.14^b	1.87±0.05^b	1.93±0.11^c	N/a
QI scores	G.	3.23±0.15^a	4.37±0.81^a	6.91±0.85^a	10.49±1.20^b	13.24±0.75^b	15.73±0.5^b	18.78±0.4^c
	U	3.3±0.45^a	6.2±0.96^a	9.1±0.75^b	12.03±0.23^b	15.6±1.05^b	18.1±0.5^c	N/a

n=9: Mean values in the same row with the same superscript are not significantly different (PÓ0.05)
Legend: G-gutted, U-ungutted

The QI scores for ungutted and gutted control Nile perch increased with storage days. Significant increase was observed from the 14th and 18th day (pÒ0.05) of storage in ice onwards for ungutted and gutted control Nile perch fish samples, respectively.

Table 4.4: QI scores for parameters of iced ungutted and gutted control Lake Victoria Nile perch (*Lates niloticus)* fish samples

Parameters		Days of sampling							
		2	6	10	14	18	22	26	28
Skin	G	0.70±0.20^{a}	0.80±0.30^{a}	0.70±0.05^{a}	1.00±0.05^{a}	1.10±0.30^{b}	1.25±0.10^{b}	1.50±0.03^{b}	1.73±0.05^{b}
	U	0.73±0.30^{a}	0.80±0.30^{a}	1.00±0.05^{a}	1.00±0.05^{b}	1.23±0.30^{b}	1.37±0.1^{b}	1.70±0.03^{b}	2.00±0.00^{c}
Slime	G	0.10±0.10^{a}	0.30±0.10^{a}	0.80±0.05^{a}	1.00±0.05^{a}	1.10±0.20^{b}	1.37±0.10^{b}	1.56±0.05^{b}	1.71±0.03^{b}
	U	0.26±0.05^{a}	0.40±0.10^{a}	1.00±0.05^{a}	1.00±0.05^{b}	1.33±0.20^{b}	1.57±0.1^{b}	1.86±0.05^{b}	2.00±0.00^{c}
Eyes Clarity	G	0.30±0.10^{a}	0.40±0.05^{a}	0.80±0.02^{a}	1.10±0.05^{a}	1.20±0.06^{b}	1.43±0.10^{b}	1.57±0.01^{b}	1.72±0.04^{b}
	U	0.46±0.05^{a}	0.47±0.05^{a}	1.00±0.02^{a}	1.27±0.05^{b}	1.27±0.06^{b}	1.63±0.1^{b}	1.77±0.01^{b}	2.00±0.00^{c}
Eyes shape	G	0.25±0.02^{a}	0.30±0.04^{a}	0.63±0.11^{a}	0.95±0.20^{a}	1.17±0.11^{a}	1.35±0.05^{b}	1.51±0.01^{b}	1.69±0.02^{b}
	U	0.20±0.02^{a}	0.57±0.04^{a}	1.33±0.11^{a}	1.27±0.20^{b}	1.37±0.11^{b}	1.50±0.05^{b}	1.77±0.01^{b}	2.00±0.00^{c}
Elasticity	G	0.21±0.05^{a}	0.30±0.10^{a}	0.70±0.10^{a}	1.07±0.11^{a}	1.30±0.05^{b}	1.43±0.01^{b}	1.61±0.22^{b}	1.71±0.02^{b}
	U	0.23±0.05^{a}	0.30±0.10^{a}	0.80±0.10^{a}	1.07±0.11^{b}	1.30±0.05^{b}	1.43±0.01^{b}	1.87±0.22^{b}	2.00±0.00^{c}
Belly	G	0.62±0.03^{a}	0.49±0.23^{a}	1.00±0.28^{a}	1.23±0.06^{b}	1.41±0.05^{b}	1.61±0.10^{b}	1.95±0.11^{b}	2.15±0.30^{c}
	U	1.34±0.03^{a}	1.49±0.23^{a}	1.63±0.28^{a}	1.73±0.06^{b}	1.86±0.05^{b}	1.98±0.10^{b}	2.05±0.11^{b}	2.17±0.30^{b}
Gill Color	G	0.43±0.25^{a}	0.47±0.23^{a}	0.63±0.11^{a}	1.00±0.23^{a}	1.27±0.15^{b}	1.31±0.05^{b}	1.53±0.25^{b}	1.69±0.04^{b}
	U	0.47±0.25^{a}	0.47±0.23^{a}	1.06±0.11^{a}	1.13±0.23^{b}	1.33±0.15^{b}	1.46±0.05^{b}	1.77±0.25^{b}	2.00±0.00^{c}
Gill Odor	G	0.21±0.05^{a}	0.53±0.05^{a}	0.82±0.15^{a}	1.23±0.15^{b}	1.41±0.20^{b}	1.53±0.40^{b}	1.93±0.30^{b}	2.25±0.11^{c}
	U	0.20±0.05^{a}	0.53±0.05^{a}	1.17±0.15^{a}	1.33±0.15^{b}	1.50±0.20^{b}	1.70±0.40^{b}	2.13±0.30^{b}	2.93±0.11^{c}
Gill Slime	G	0.25±0.05^{a}	0.41±0.17^{a}	0.71±0.05^{a}	1.00±0.15^{a}	1.21±0.06^{b}	1.45±0.20^{b}	1.60±0.11^{b}	1.73±0.02^{b}
	U	0.27±0.05^{a}	0.40±0.17^{a}	1.00±0.05^{a}	1.20±0.15^{b}	1.27±0.06^{b}	1.70±0.20^{b}	1.93±0.11^{c}	2.00±0.02^{c}
QI scores	G	3.07±0.34^{a}	4.0±0.33^{a}	6.79±0.44^{a}	9.58±0.17^{a}	11.17±0.47^{b}	12.73±0.23^{b}	14.7±0.22^{b}	16.4±0.1^{b}
	U	3.37±0.78^{a}	4.53±0.83^{a}	9.30±0.44^{a}	10.5±0.17^{b}	12.13±0.47^{b}	14.33±0.65^{b}	16.6±0.26^{b}	19.0±0.10^{c}

n=9 Mean values in the same row with the same superscript are not significantly different (pÓ0.05) Legend: G-gutted, U-ungutted

There was increase in the demerit points of the individual sensory parameters for ungutted control Nile perch fish samples over storage period in ice (Figure 4.1)

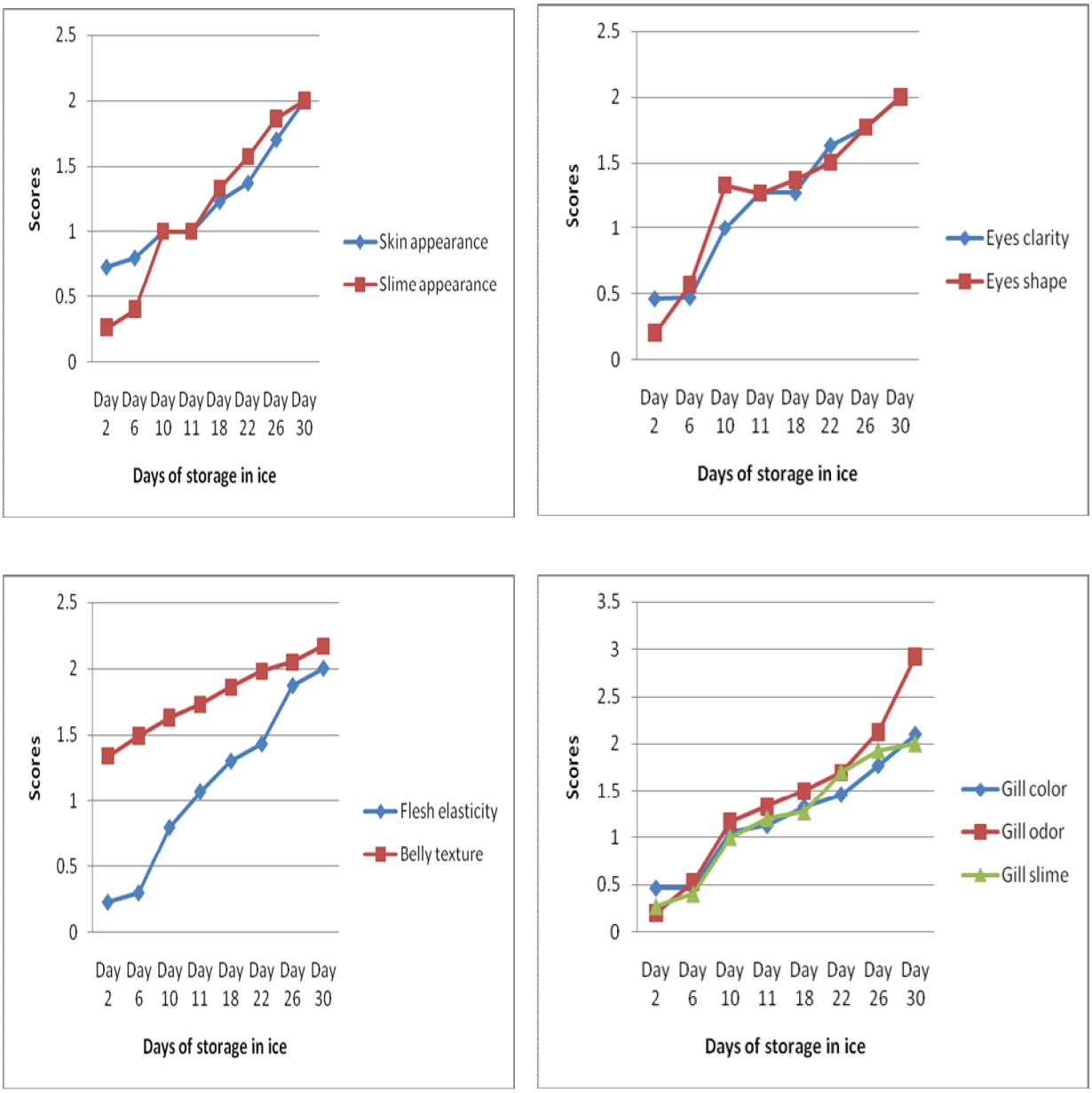

Figure. 4.1: Demerit points of each quality attribute for ungutted control fish samples assessed with the Quality Index Method over storage days in ice.

Figure 4.2 shows linear relationship between the QI scores and the storage time in ice for ungutted control fish samples. This could be used as calibration curve for predicting remaining storage life of Nile perch in ice.

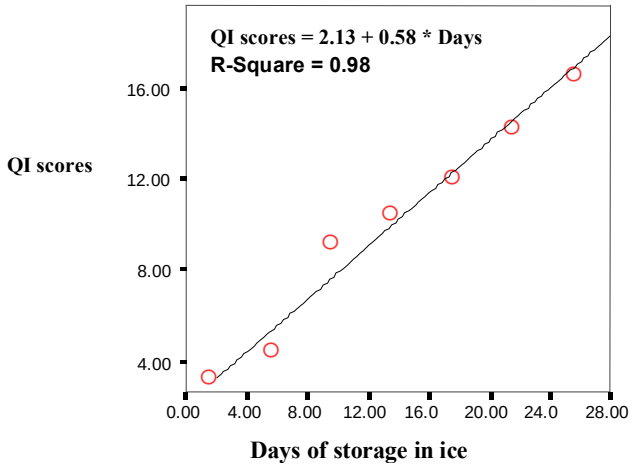

Figure 4.2: Average QI scores over storage time of ungutted control Nile perch stored in ice

4.1.2 Sensory evaluation of cooked fish

This was done to predict the shelf life of Nile perch. The hedonic rating for organoleptic properties of cooked gutted and ungutted experimental and control Nile perch fish fillet over the period of storage in ice are shown in Table 4.5 and Table 4.6. Score of Ò4.0 for the organoleptic properties was considered the rejection threshold level for the fish.

Table 4.5: Mean scores for organoleptic properties of ungutted and gutted experimental cooked fillet samples

Days of Sampling	Odour		Taste		Texture	
	Ungutted	Gutted	Ungutted	Gutted	Ungutted	Gutted
2	8.30±0.12^{a}	8.90±0.11^{a}	8.30±0.20^{a}	9.00±0.31^{a}	8.70±0.50^{a}	9.00±0.45^{a}
6	8.00±0.20^{a}	8.50±0.30^{a}	8.00±0.40^{a}	8.70±0.50^{a}	7.83±0.55^{a}	8.60±0.63^{a}
10	7.70±0.12^{a}	8.00±0.41^{a}	7.40±0.47^{a}	8.00±0.30^{a}	7.30±0.26^{a}	8.10±0.32^{a}
14	7.50±0.61^{b}	7.50±0.33^{a}	7.20±0.52^{b}	7.50±0.24^{a}	6.40±0.51^{b}	7.60±0.35^{a}
18	5.90±0.31^{b}	6.80±0.31^{b}	6.10±0.12^{b}	6.70±0.12^{b}	5.20±0.72^{b}	6.70±0.22^{b}
22	4.40±1.24^{c}	5.00±0.44^{b}	4.30±0.79^{c}	5.30±0.55^{b}	3.60±0.47^{c}	5.40±0.27^{b}
26	N/a	4.0±0.11^{c}	N/a	3.6±0.22^{c}	N/a	3.3±0.30^{c}
28	N/a	N/a	N/a	N/a	N/a	N/a

n=9; Mean values in the same column with the same superscript are not significantly different (pÓ0.05)

N/a- No results for the parameters were given due to the arrival of end of shelf life

Table 4.6: Mean scores for organoleptic properties of ungutted and gutted control cooked fillet samples

Days of	Odour		Taste		Texture	
Sampling	Ungutted	Gutted	Ungutted	Gutted	Ungutted	Gutted
2	9.2±0.1[a]	9.2±0.1[a]	9.6±0.2[a]	9.4±0.1[a]	9.2±0.4[a]	9.2±0.2[a]
6	8.8±0.2[a]	9.0±0.1[a]	8.8±0.3[a]	9.2±0.2[a]	8.8±0.1[a]	9.0±0.2[a]
10	8.4±0.3[a]	8.6±0.2[a]	8.8±0.2[a]	8.6±0.3[a]	8.4±0.3[a]	8.6±0.2[a]
14	7.6±0.2[a]	8.0±0.2[a]	7.2±0.3[a]	8.2±0.2[a]	8.0±0.3[a]	8.0±0.4[a]
18	7.2±0.2[b]	7.6±0.2[a]	6.8±0.2[b]	7.8±0.1[a]	7.6±0.2[b]	7.6±0.2[a]
22	6.0±0.1[b]	7.2±0.3[b]	6.0±0.2[b]	7.4±0.2[a]	5.7±0.1[b]	7.2±0.1[b]
26	4.7±0.2[b]	6.8±0.2[b]	4.5±0.1[c]	7.0±0.1[b]	4.4±0.2[b]	6.6±0.2[b]
28	3.7±0.1[c]	6.0±0.2[b]	3.4±0.1[c]	6.0±0.2[b]	3.1±0.1[c]	6.0±0.2[b]

n=9; Mean values in the same column with the same superscript are not significantly different (pÓ0.05)

N/a- No results for the parameters were given due to the arrival earlier of the end of shelf life

4.2 Microbiological parameters and chemical parameters

There was increase in the microbiological and biochemical parameters of ungutted and gutted experimental Nile perch fish samples over the storage period in ice. Significant increase was observed on the 10^{th} and 14^{th} day (pÒ0.05) of storage in ice for ungutted and gutted experimental fish samples, respectively (Table 4.7).

Table 4.7: Trend in the microbiological and biochemical parameters analyzed for gutted and ungutted experimental Nile perch

Parameters		Days of sampling						
		2	6	10	14	18	22	26
TVC log cfu/g at 22ºC	G	2.7±0.23^{a}	3.3±0.21^{a}	3.9±0.42^{a}	4.4±0.35^{b}	5.0±0.32^{b}	5.3±0.11^{c}	5.9±0.19^{d}
	U	4.0±0.14^{a}	4.9±0.32^{a}	5.3±0.25^{b}	5.9±0.24^{b}	6.3±0.24^{c}	7.0±0.11^{e}	N/a
TVC log cfu/g at 37ºC	G	2.1±0.11^{a}	2.9±0.45^{a}	3.2±0.44^{a}	4.0±0.27^{b}	4.4±0.17^{b}	5.0±0.28^{c}	5.4±0.21^{d}
	U	3.1±0.17^{a}	4.3±0.27^{a}	5.0±0.22^{b}	5.5±0.37^{b}	5.9±0.40^{c}	6.7±0.33^{d}	N/a
H_2S log cfu/g at 22°C	G	2.5±0.43^{a}	3.0±0.44^{a}	3.7±0.33^{a}	4.3±0.17^{b}	4.7±0.28^{b}	5.1±0.22^{c}	5.5±0.27^{d}
	U	3.3±0.25^{a}	4.5±0.15^{a}	4.9±0.17^{b}	5.3±0.26^{b}	6.0±0.45^{c}	6.50±0.55^{d}	N/a
Coliforms	G	1.3±0.32^{a}	1.5±0.22^{a}	1.5±0.26^{a}	1.5±0.12^{a}	1.5±0.30^{a}	1.8±0.37^{a}	1.7±0.13^{a}
	U	2.7±0.34^{a}	3.3±0.33^{a}	3.9±0.10^{a}	4.3±0.15^{b}	4.7±0.55^{b}	5.1±0.32^{c}	N/a
E.coli	G	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	U	Present	Present	Present	Present	Present	Present	N/a
Salmonella	G	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	U	Absent	Absent	Absent	Absent	Absent	Absent	N/a
pH	G	6.85±0.05^{a}	6.85±0.05^{a}	6.91±0.02^{a}	6.93±0.07^{a}	6.97±0.05^{b}	7.05±0.04^{b}	7.11±0.05^{b}
	U	6.85±0.05^{a}	6.85±0.05^{a}	6.94±0.01^{a}	7.01±0.05^{b}	7.08±0.01^{b}	7.18±0.04^{b}	N/a
FFA %	G	1.90±0.00^{a}	1.90±0.00^{a}	2.11±0.03^{a}	2.24±0.05^{a}	2.41±0.06^{b}	2.51±0.10^{b}	2.59±0.11^{b}
	U	1.9±0.00^{a}	1.9±0.00^{a}	2.11±0.02^{a}	2.38±0.05^{b}	2.47±0.06^{b}	2.66±0.10^{b}	N/a
TVBN mgN/100g	G	8.4±0.10^{a}	8.40±0.50^{a}	9.13±0.51^{a}	13.90±0.71^{b}	15.24±0.14^{b}	21.00±0.6^{c}	24.52±0.23^{c}
	U	8.4±0.7^{a}	9.33±0.4^{a}	12.83±0.4^{b}	16.57±1.0^{b}	21.93±1.4^{c}	26.03±0.9^{c}	N/a
TMAO	G	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	U	Nil	Nil	Nil	Nil	Nil	Nil	N/a
TMAN	G	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	U	Nil	Nil	Nil	Nil	Nil	Nil	N/a

n=9 Mean values in the same row with the same superscript are not significantly different (pÓ0.05)
Legend: G- gutted, U-ungutted

There was increase in the microbiological and biochemical parameters of ungutted and gutted control Nile fish samples over the storage period in ice. Significant increase was observed on the 14th and 18th day (pÒ0.05) of storage in ice for ungutted and gutted experimental fish samples, respectively (Table 4.8).

Table 4.8: Trend in the microbiological and biochemical parameters for iced gutted and ungutted control Nile perch

Parameters		**Days of sampling**							
		2	6	10	14	18	22	26	28
TVC log cfu/g at 22ºC	G	2.5±0.23^{a}	2.7±0.11^{a}	4.5±0.14^{a}	4.9±0.32^{a}	5.3±0.15^{b}	5.5±0.25^{b}	5.8±0.37^{c}	6.0±0.23^{c}
	U	2.5±0.32^{a}	3.3±0.22^{a}	4.7±0.50^{a}	5.1±0.23^{b}	5.4±0.24^{b}	5.8±0.22^{c}	6.0±0.22^{c}	6.4±0.14^{d}
TVC log cfu/g at 37ºC	G	2.0±0.14^{a}	2.1±0.23^{a}	3.6±0.15^{a}	4.3±0.35^{a}	4.8±0.14^{b}	5.1±0.28^{b}	5.3±0.33^{c}	5.9±0.22^{c}
	U	2.0±0.21^{a}	2.5±0.13^{a}	3.7±0.17^{a}	4.7±0.14^{b}	5.0±0.35^{b}	5.5±0.33^{c}	6.1±0.21^{d}	6.3±0.25^{d}
H_2S log cfu/g at 22°C	G	1.2±0.10^{a}	2.1±0.24^{a}	2.5±0.32^{a}	2.9±0.23^{a}	3.9±0.12^{b}	4.5±0.34^{c}	4.9±0.28^{c}	5.1±0.27^{d}
	U	1.4±0.11^{a}	2.4±0.21^{a}	3.5±0.15^{a}	3.8±0.23^{a}	4.5±0.37^{b}	5.2±0.13^{c}	5.6±0.32^{d}	6.0±0.51^{e}
Coliforms cfu/g	G	1.7±0.15^{a}	1.2±0.21^{a}	1.5±0.41^{a}	1.6±0.22^{a}	1.7±0.14^{a}	1.7±0.10^{a}	1.8±0.11^{a}	2.0±0.41^{a}
	U	2.6±0.20^{a}	3.2±0.26^{a}	3.6±0.32^{a}	4.2±0.34^{b}	4.8±0.21^{b}	5.1±0.22^{b}	5.6±0.15^{c}	5.8±0.56^{c}
E. coli	G	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	U	Present	Absent	Present	Present	Absent	Absent	Present	Absent
pH	G	6.80±0.00^{a}	6.85±0.05^{a}	6.85±0.02^{a}	6.88±0.2^{a}	6.91±0.01^{a}	6.97±0.03^{b}	7.00±0.04^{b}	7.04±0.02^{b}
	U	6.84±0.05^{a}	6.83±0.05^{a}	6.91±0.01^{a}	6.95±0.1^{a}	6.99±0.01^{b}	7.04±0.05^{b}	7.08±0.03^{b}	7.14±0.05^{b}
FFA %	G	1.9±0.02^{a}	1.9±0.03^{a}	2.0±0.04^{a}	2.1±0.03^{a}	2.4±0.07^{b}	2.5±0.05^{b}	2.61±0.03^{b}	2.65±0.2^{b}
	U	1.9±0.05^{a}	1.9±0.05^{a}	2.1±0.05^{a}	2.3±0.05^{a}	2.4±0.06^{b}	2.5±0.04^{b}	2.60±0.02^{b}	2.72±0.5^{b}
TVBN mgN/100g	G	8.4±0.05^{a}	8.4±0.05^{a}	11.2±0.3^{a}	12.6±0.5^{b}	14.0±0.7^{b}	15.4±0.5^{b}	18.2±0.6^{c}	21.0±0.5^{c}
	U	8.4±0.05^{a}	8.63±0.4^{a}	11.63±0.4^{a}	15.4±0.4^{b}	16.56±0.9^{b}	17.97±0.4^{b}	21.93±0.8^{c}	26.83±0.4^{c}
TMAO mgN/100g	G	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	U	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
TMAN mgN/100g	G	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	U	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

n=9,Mean values in the same row with the same superscript are not significantly different (pÓ0.05) Legend: G- gutted, U-ungutted

4.3 Proximate composition

Table 4.9 shows the trend in the proximate composition of protein, lipid, moisture and ash gutted and ungutted experimental and control iced Nile perch fish samples over the storage period in ice. There was no significant (pÓ0.05) increase in the lipid, moisture and ash contents for all the fish samples. The protein contents decreased with increase in storage days. Significant decrease (pÒ0.05) was observed on the 18th day for the ungutted fish samples and on the 22nd day for gutted fish samples, respectively.

Table 4.9: Trend in the proximate composition of iced experimental and control Nile perch fish samples

Parameters		Days of sampling							
		2	6	10	14	18	22	26	28
Moisture %	G.E	78.61±0.35^a	78.63±0.25^a	78.17±0.17^a	78.43±0.39^a	78.70±0.31^a	78.56±0.11^a	78.5±0.25^a	N/a
	U.E	78.5±0.36^a	78.53±0.35^a	79.07±0.37^a	79.33±0.49^a	79.56±0.31^a	79.56±0.21^a	N/a	N/a
	G.C	78.5±0.2^a	78.3±0.03^a	78.5±0.2^a	78.6±0.1^a	78.5±0.1^a	78.6±0.3^a	78.5±0.1^a	78.5±0.4^a
	U.C	78.4±0.36^a	78.6±0.05^a	78.2±0.7^a	78.7±0.2^a	78.7±0.5^a	79.56±0.21^a	79.00±0.2^a	78.70±0.4^a
Ash %	G.E	0.63±0.06^a	0.62±0.02^a	0.60±0.04^a	0.61±0.03^a	0.63±0.01^a	0.59±0.05^a	0.60±0.06^a	N/a
	U.E	0.63±0.05^a	0.60±0.03^a	0.59±0.02^a	0.6±0.02^a	0.59±0.01^a	0.55±0.05^a	N/a	N/a
	G.C	0.61±0.04^a	0.60±0.03^a	0.61±0.01^a	0.62±0.02^a	0.62±0.03^a	0.62±0.03^a	0.61±0.04^a	0.61±0.03^a
	U.C	0.62±0.05^a	0.61±0.02^a	0.61±0.05^a	0.6±0.02^a	0.59±0.02^a	0.61±0.01^a	0.60±0.05^a	0.61±0.02^a
Crude Lipids %	G.E	0.63±0.01^a	0.60±0.01^a	0.63±0.02^a	0.60±0.00^a	0.61±0.02^a	0.62±0.05^a	0.61±0.02^a	N/a
	U.E	0.64±0.01^a	0.60±0.01^a	0.61±0.01^a	0.60±0.02^a	0.59±0.01^a	0.61±0.07^a	N/a	N/a
	G.C	0.61±0.02^a	0.64±0.03^a	0.62±0.05^a	0.63±0.02^a	0.61±0.01^a	0.61±0.05^a	0.62±0.04^a	0.63±0.02^a
	U.C	0.63±0.05^a	0.63±0.01^a	0.62±0.06^a	0.61±0.02^a	0.6±0.01^a	0.58±0.44^a	0.57±0.08^a	0.60±0.01
Crude proteins %	G.E	19.83±0.11^a	19.11±0.31^a	19.0±0.2^a	18.72±0.12^a	18.46±0.15^a	18.23±0.11^b	18.00±0.12^b	N/a
	U.E	19.53±0.12^a	19.23±0.21^a	19.0±0.1^a	18.47±0.21^a	18.10±0.17^b	17.73±0.21^b	N/a	N/a
	G.C	19.5±0.1^a	19.3±0.1^a	18.90±0.21^a	18.8±0.1^a	18.4±0.14^a	18.2±0.15^b	18.1±0.1^b	18.0±0.2^b
	U.C	19.53±0.15^a	19.23±0.12^a	18.9±0.2^a	18.8±0.2^a	18.43±0.17^b	18.13±0.15^b	17.9±0.16^b	17.5±0.10^b

n=9Mean values in the same row with the same superscript are not significantly different (pÓ0.05) Legend: G.E- gutted experimental samples, G.C- gutted control samples, U.E- ungutted experimental samples, U.C- ungutted control samples.

Table 4.10 shows the mean coliform counts for fishing ground, boat, shore and tap water. There was no significant difference (pÓ0.05) between the boat and shore water while significant difference (pÒ0.05) was observed between the fishing ground and tap water and boat and shore water.

Table 4.10: Mean coliform counts at 37°C for water samples

Source	**Log_{10} MPN per mL**
Fishing ground water	2.5 ± 0.21^{a}
Boat water	$>3.38\pm0.00^{b}$
Shore water	$>3.38\pm0.00^{b}$
Tap water	1.8 ± 0.18^{c}

Mean values in the same column with the same superscript are not significantly different (pÓ0.05)

4.4 Correlation between the indices

Table 4.11 shows the correlation among the sensory, chemical and microbiological indices

Table 4.11: Correlations between the different indices studied

Indices	pH	TVBN	FFA	QI scores	TVC	H_2S
pH	-	0.98	0.96	0.97	0.81	0.74
TVBN	0.98	-	0.98	0.97	0.82	0.75
FFA	0.96	0.98	-	0.96	0.81	0.69
QI scores	0.97	0.97	0.96	-	0.93	0.98
TVC	0.81	0.82	0.81	0.93	-	0.90
H_2S	0.74	0.75	0.69	0.98	0.90	-

Legend: H2S- Hydrogen sulphide producing bacteria. Correlations are significant at pÒ0.01

Figure 4.3 and 4.4 show linear relationship between the QI scores and the TVC and H_2S producing bacteria, respectively.

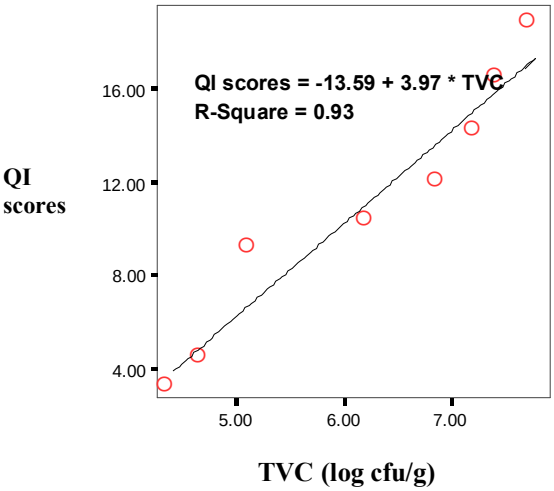

Fig. 4.3. Relationship between TVC and QI scores of control Nile perch fish stored in ice

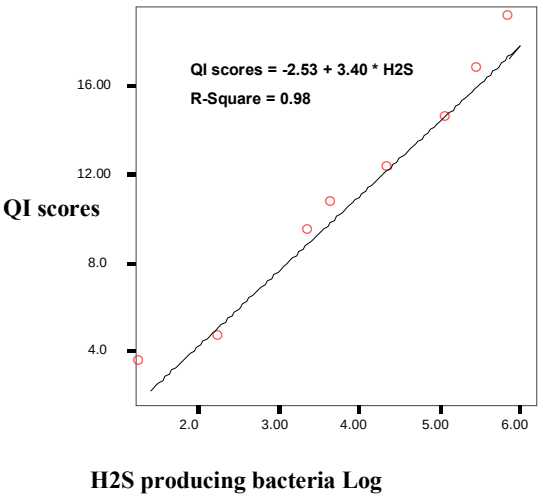

Figure 4.4: Relationship between H2S producing bacteria and QI scores of control Nile perch fish stored in ice

CHAPTER FIVE
DISCUSSION

5.1 Sensory Analysis

5.1.1 The quality index scheme

The initial parameters developed for the preliminary scheme included the appearance of the skin and slime, eyes clarity and shape, flesh color in open surfaces, adherence of the peritoneum, texture (flesh elasticity and belly texture), and the colour, odour of gills and gill slime. These parameters were assigned scores of between 0 and 3. The initial maximum sum of demerit points was 24 (Table 4.1). This scheme was applied to evaluate a batch of Nile perch for a period of 30 days. During the development of the scheme, the parameters flesh colour in open surfaces and the adherence of the peritoneum were removed from the preliminary scheme because they were difficult to evaluate and failed to reach the assigned maximum of 1 demerit point at the time the cooked fish reached the rejection threshold. The scores range assigned for skin appearance was also reduced by 1 demerit point to 2 because the black colour described in the preliminary scheme could not be reached at the end of the storage time. It was also found that on day 30, the ungutted control fish samples were unfit for human consumption and therefore the researcher decided to store the fish up to day 28 and sample on both the 26^{th} and 28^{th} day for the sake of greater accuracy in assessing the end of shelf life of the fish in ice.

The results, comments and suggestions obtained from the panelists during the initial assessment using the preliminary scheme reduced the parameters from an initial number of 11 to 9 representing a total of 20 demerit points (Table 4.2). This final scheme was used to assess the fish for a storage period of 28 days. The sum of the scores in the QIM was designated as the Quality Index (QI) score.

There was an increase in the QI scores attributes with storage time in ice for both ungutted and gutted experimental (Table 4.3) and control fish samples (Table 4.4). The highest QI scores for all the parameters for the ungutted experimental and control fish samples was reached on day 22 and day 28 of storage in ice, respectively, at which point the fish was considered unfit for human consumption. The same was observed for the gutted experimental and control fish samples on day 26 and day 28 of storage in ice, respectively, although at this point only the ungutted experimental fish samples were unfit for human consumpttion. Gutting increased the shelf life of the Nile perch bought at the beaches by approximately 4 days.

The QI scores for the individual quality attributes in the QIM scheme increased with storage time (Fig. 4.1). The skin appearance was very bright and pearl shiny on day 2 of storage in ice and this changed to dull on the 28th day. The skin slime was generally clear and transparent on day 2 progressing to a cloudy appearance on day 28. The eyes clarity was initially clear and translucent but changed to opaque on day 28. This corresponded to the changes in the shape of the eyes from convex to concave. The skin texture could be described as elastic on day 2 but became clearly marked by pressure on day 28. This was also observed for the belly texture which was intact at the beginning of storage but very soft at the end of storage. Gill odour was very fresh and seaweedy on day 2 of storage in ice, changing to off-odour or rotten odour on day 28 of storage in ice. These changes were also observed for gill slime which changed from a clear-translucent appearance to cloudy appearance on day 28. It was possible to quantify the changes described above into QI scores. These changes in physical characteristics are given in Appendix 2.

The QI scores increased with storage time in ice. This is in accordance with how the QIM scheme is constructed, where iced fish evaluated after catch should be scored low and the scores subsequently increasing with storage time, reaching close to maximum score at the end of the shelf life (Martinsdottir *et al.*, 2001). The increase in the scores for the sensory parameters considered in the QIM scheme shows loss of freshness hence deterioration in fish quality. It was apparent from this study that the slime, elasticity, gill colour and gill odour determined the sensory quality of this fish since the scores given by the panel for these parameters were in most cases more than half the total value expected at the point when a significant increase in the microbiological and chemical parameters was detected. The high correlation (R^2 = 0.98) of the QI scores with the storage time for iced Nile perch implies that the QIM scheme reflected the deteriorative changes in Nile perch with storage time accurately (Figure 4.2). It can be explained that the QIM scheme developed makes it possible to determine the freshness of Nile perch at any particular storage time and even predict the remaining storage life of this fish in ice. This is shown in the calibration curve presented in Figure 4.2.

These observed changes may be due to the combined effect of biochemical and microbial actions during autolysis influenced by both fish muscle enzymes and microbial enzymes as was also observed by Gill (1995) and Nielsen (1995). The changes quantified as QI scores had a high correlation with TVC (0.93) (Figure 4.3) and H_2S producing bacteria (0.98) (Figure 4.4). It may

be explained that the increase in the TVC and H_2S producing bacterial counts could have enhanced a breakdown of nitrogenous compounds in the fish flesh during their metabolism. This is because the bacteria were able to rapidly and effectively utilize the non protein nitrogen compounds that constituted the available substrate for nutrition. The oxidative deamination of amino acids followed by the re-incorporation into the fish muscle of released ammonia to serve as the nitrogen source is the primary mechanism involved in these changes (Ward, 1994). This may have led to the accumulation of volatile amines and free fatty acids and can explain the corresponding increase in the levels of TVBN and FFA with storage time in ice (Table 4.7 and 4.8). The accumulation of these volatile amines and FFA were therefore responsible for the off-odour, textural and flavour changes observed as storage time in ice progressed.

The QI scores for the individual quality attributes for gutted, washed and iced fish also increased with storage time although the score values at any particular sampling time were lower than the QI scores for corresponding ungutted experimental and control fish samples (Table 4.3 and 4.4). The lower QI scores for the gutted, washed and iced fish samples could be attributed to slower microbiological and biochemical activity as a result of the removal of the gut bacteria and washing which could have reduced the microbial load on the fish muscle (Nielsen, 1995). This resulted in reduced rate of utilization of non protein nitrogen compounds that constituted the available substrate for nutrition. The bacterial rate of decomposition of nitrogenous compounds was reduced hence leading to a slightly slower rate of accumulation of volatile amines and free fatty acids. This partly explains the corresponding lower levels of TVBN and FFA with storage time in ice as compared to those of ungutted fish samples (Table 4.7 and 4.8).

5.1.2 Sensory evaluation of cooked Nile perch fillets

The hedonic rating for the odour, flavour and texture during the sensory evaluation of cooked ungutted experimental (Table 4.5) and control fish samples (Table 4.6) significantly decreased ($p<0.05$) with storage time in ice from the 14^{th} day and 18^{th} day onwards, respectively. The high scores observed on day 2 of storage showed the odour of the cooked fish was fresh to seaweedy while the taste was sweet with a characteristic meaty flavour. The texture was firm. At the point of rejection, there was a strong ammoniacal smell, the taste was sour, fishy and musty, while the flesh texture was very soft and mealy. The development of these spoilage characteristics can be attributed to the combined effects of chemical and bacterial activity. The hydrolytic rancidity which originates from lipases producing free fatty acids (FFA) which undergo further oxidation

to produce low molecular weight compounds may have been responsible for the above off-flavours as was also observed by Olafsdottir *et al.* (1997) and Shewan (1979). The development of these sensory characteristics also corresponded with the increase in the level of bacterial counts (TVC and H_2S producing bacteria) (Tables 4.7 and 4.8) which may have been responsible for the breakdown of non protein compounds in the muscle to volatile amines and accumulation of FFA.

The hedonic scores for odour, flavour and texture during sensory evaluation for cooked gutted experimental (Table 4.5) and control (Table 4.6) fish samples also significantly decreased ($p \leq 0.05$) with storage time from the 18^{th} and 22^{nd} day of storage onwards, respectively. The scores of these parameters were slightly higher than those scored for the corresponding ungutted fish samples at any given sampling time. At the point of rejection, which was on day 26 of storage for the gutted experimental fish samples, there was a strong ammoniacal smell, the taste was sour, fishy and musty, while the flesh texture was very soft and mealy. The gutted control fish samples, had slightly fishy odour and taste with less firm texture on day 28 of storage in ice, which was represented by an average score of 6.0 for odour, flavour and texture (Table 4.6). It may be explained that the gutting and washing process may have reduced the microbial load hence reducing the rate of hydrolytic rancidity which originates from the lipases to produce free fatty acids. The rate of oxidation to produce low molecular weight compounds therefore resulted in a slightly slower rate of development of these spoilage characteristics. This is supported by corresponding lower levels of TVC and H_2S bacterial counts compared to the levels of these parameters in the ungutted experimental and control fish samples (Table 4.7 and 4.8).

5.2 Biochemical parameters

5.2.1 pH

The pH is the negative logarithm of hydrogen ion concentration. The pH is one of the factors that determines the survival and growth of micro-organisms during storage, processing and distribution of fish. The pH of live fish muscle is normally close to the value 7.0. However, post mortem pH varies from 6.0 to 7.1 (Pacheco-Aguilar *et al.*, 2000).

The pH of the ungutted experimental (Table 4.7) and control (Table 4.8) Nile perch fish samples significantly increased ($p \leq 0.05$) with storage time from the 14^{th} and 18^{th} day onwards, respectively. On day 22 and 28 the ungutted fish samples bought from the beaches and those

immediately iced after removal from the net were considered unacceptable for human consumption, respectively. The pH was 7.18 and 7.14, respectively.

The pH of fish flesh has an important influence on its freshness because of its influence on bacterial growth. At pH < 4.5 the bacteria that is responsible for food spoilage are gram positive such as *Lactobacillus spp.* while at pH >6 gram negative bacteria dominate such as *Pseudomonas spp* (Ward, 1994). Increases in pH may indicate the accumulation of volatile amines such as ammonia mainly derived from microbial actions through bacterial degradation or deamination of proteins, peptides and amino acids in the fish muscle. The increase may also be due to an increase in volatile bases from the decomposition of nitrogenous compounds by endogenous or microbial enzymes (Erkan and Ozden, 2008). The increase in the volatile bases which therefore increased the pH formed a favourable environment for the growth of alkaline tolerant micro-organisms which are mostly gram-negative bacteria. This corresponded with the increase in the levels of TVC, H_2S producing bacteria, coliforms, and TVBN (Table 4.7 and 4.8).

The pH of the gutted, washed and iced experimental (Table 4.7) and control (Table 4.8) fish samples significantly increased (pÓ0.05) from 14th and 18th day of storage in ice, respectively. On day 26 and 28 the pH was 7.11±0.05 and 7.00±0.04 for the gutted experimental and control fish samples, respectively. There were significantly lower (pÒ0.05) levels of pH levels for gutted, washed and iced fish compared to the pH levels of the ungutted fish samples at every sampling time (Table 4.7 and 4.8). It may be explained that the gutting and washing of fish samples had a significant effect on pH levels by reducing the microbial levels and hence reducing the rate of degradation or deamination of proteins, peptides and amino acids. This therefore led to the slower rate of accumulation of volatile bases such as ammonia from the decomposition of nitrogenous compounds by endogenous and microbial enzymes. The resultant effect is the slightly slower rate of increase in the levels of TVC, H_2S producing bacteria, and TVBN.

5.2.2 TVBN

The TVBN of ungutted experimental and control fish samples showed an increase with storage time in ice. There was a significant (pÒ0.05) increase in the values of this index from the 10th and 14th day of storage in ice for the ungutted experimental (Table 4.7) and control (Table 4.8) fish samples, respectively. This significant increase (pÒ0.05) 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 resultant increase in the levels of TVBN could therefore be responsible for the spoilage characteristics observed at this particular time (Table 4.5 and 4.6).

The formation of TVBN is associated with the activity of micro-organisms and tends to be high at high microbial populations as observed by Benjakul *et al.* (2003) and Chytiri *et al.* (2004). The TVBN for ungutted experimental and control fish samples was 26.03 mgN/100 g flesh and 26.83±0.04 mgN/100 g flesh, respectively, at the time when the cooked fish was considered unfit for human consumption. At this point, the TVC and H_2S bacterial counts for the ungutted experimental and control fish samples were Ó 6 log cfu/g. There were also high pH values of 7.14 ± 0.05 and 7.18 ±0.04 for the experimental and control fish samples, respectively. Since TVBN is produced mainly by bacterial decomposition of proteins in fish flesh, the high values of TVC and H_2S bacterial counts on the 26th and 28th day for the ungutted experimental and control fish samples, respectively, could explain the considerable levels of TVBN in Nile perch flesh at this time of storage. The initial lower levels of TVBN observed could be attributed to lower levels of endogenous ammonia due to slower microbial breakdown of proteins during the first 10 and 14 days of storage in ice (Pacheco-Aguilar *et al.,* 2000). These results followed similar trends with other studies of the L. Victoria Nile perch as observed by Gram *et al.* (1987) and Karungi *et al.* (2004).

The TVBN levels for gutted, washed and iced experimental and control fish samples also increased with storage time in ice. These levels increased significantly (pÒ0.05) on the 14th and 18th day of storage for the gutted experimental (Table 4.7) and control (Table 4.8) fish samples, respectively. On the 26th and 28th day of storage in ice, the TVBN levels were 24.52 mgN/100 g flesh and 21.0±0.5 mgN/100 g flesh, respectively. These levels were less than the levels of TVBN noted for ungutted experimental and control fish samples. It may be explained that the levels of TVBN in gutted, washed and iced fish samples were much lower due to reduced gut microbial load and hence reduced bacterial decomposition of nitrogenous compounds in the fish flesh (Ozogul *et al.*, 2004). This relationship may be supported by the lower levels in the TVC and H_2S producing bacterial counts compared to the corresponding levels of these microbial parameters for the ungutted fish samples (Table 4.7 and 4.8).

5.2.3 TMAO and TMAN

Trimethylamine oxide is an osmoregulatory compound in marine teleosts. The absence of trimethylamine oxide in both ungutted and gutted experimental and control Nile perch fish

samples from fresh water could be an indication that this non protein nitrogen component was non existent in the fish or existed in amounts that could not be detected by the method used. TMAO had been detected in fresh Nile perch fish samples at levels of 11 mgN/100g and 35 mgN/100g for Nile perch fish stored in ice (Gram *et al*, 1989). This study therefore differs with the earlier study.

The absence of TMAO would have resulted into the absence of TMAN since TMAN is a product of bacterial reduction of TMAO. Alternatively, it may be as a result of the absence of the well specific spoilage bacteria which were able to utilize TMAO as the terminal electron acceptor in anaerobic respiration resulting in TMAN.

5.2.4 Free Fatty Acids (FFA)

Hydrolysis of lipids in fish during chill storage normally results in the release of free fatty acids (FFA). This is catalysed by lipases which are digestive enzymes found in the fish gut. There was a significant increase ($p \leq 0.05$) in the FFA content of ungutted experimental (Table 4.7) and control (Table 4.8) fish samples from the 14th day of storage in ice onwards. The levels corresponded with an increase in the QI scores, TVC, H_2S producing bacteria TVBN and pH. Lipid hydrolysis which was catalysed by lipases and phospholipases occurred to a great extent at the end of the storage period. This could be attributed to increased lipase and phospholipase production from Nile perch digestive organs, which could have been released into the Nile perch fish muscle (Pacheco-Aguillar *et al.,* 2000). In addition, extracellular lipases produced by certain gram negative bacteria may have also contributed to lipolysis in Nile perch tissue (Nayak *et al.*, 2003). This is demonstrated by the sour, fishy, musty and rancid flavour at the points of rejection (Table 4.5 and 4.6)

The levels of FFA for gutted, washed and iced experimental (Table 4.7) and control (Table 4.8) fish samples also increased significantly ($p \leq 0.05$) from the 18th day of storage in ice onwards. On the 26th day when the experimental gutted fish samples were considered unfit for human consumption, the FFA content was 2.59% while the FFA content for control gutted fish samples was 2.65% on the 28th day of storage in ice. It may be explained that the gutting and washing process reduced the levels of bacteria. This contributed to the lower levels of FFA through reduced production of digestive enzymes-lipases and phospholipases, hence decelerating the rate of lipid hydrolysis. The washing process may also have reduced the levels of certain

micro-organisms that are responsible for the production of extracellular lipases that catalyze lipid hydrolysis as had earlier been observed by Ward (1994) and Papadopoulas *et al*. (2003). This is supported by corresponding significantly (pÒ0.05) lower levels of TVC, H_2S producing bacteria counts (Table 4.7 and 4.8).

5.3 Microbiological parameters

5.3.1 Total Viable Count and hygiene indicator micro-organisms

The ungutted experimental (Table 4.7) fish samples had significantly (pÒ0.05) higher levels of TVC and coliform counts than the ungutted control fish samples (Table 4.8). The Kenya standard for TVC in fresh fish is Ò6 log cfu/g (KEBS, 2001). The external sources that influence the increase in the TVC and coliform counts are water, personnel and equipment. Normally in the early storage days of newly caught fish the flesh is sterile probably because the immune system of the live and newly harvested fish prevents bacteria from growing. However, when the fish dies, the immune system collapses and consequently, during storage, bacteria invade the flesh resulting in high bacterial growth and total counts. It may be explained that the high TVC for the ungutted experimental fish samples could be attributed to the unhygienic environment in which the fish was handled and the longer delay before icing. This can be supported by the higher coliform counts that were recorded for the water samples that were taken from the shores and the boats (Table 4.10).

The nutritional components of fish samples provided a favourable environment for the growth of bacteria since they were able to breakdown the nitrogenous compounds for their nutrition hence the increase in numbers with the storage time. The breakdown of nitrogenous nutritional substrates for the bacteria into volatile amines raised the post mortem pH resulting in an increase in alkaline tolerant bacteria with storage time in ice. This is also supported by the increase in the levels TVBN and the high correlation between the TVC and the pH (0.81) and TVBN (0.82) (Table 4.11). The increase in the bacterial counts also manifested itself in the changes in the sensory characteristics resulting in an increase in the demerit points. The correlation between the TVC and the demerit points was high (r^2 = 0.93) indicating that the microbial growth could have contributed to the observed sensory changes (Fig.4.3).

There was significant increase (pÒ0.05) in the mesophilic counts for the ungutted experimental (Table 4.7) and control (Table 4.8) fish samples on the 10^{th} and 14^{th} day of storage

in ice onwards. The initial microbial load of freshwater fish varies depending on water conditions and temperature. Most available literature on different freshwater fish species (tilapia, striped bass, rainbow trout, silver perch) reports bacterial counts of 10^2ï 10^6 cfu/g (Acuff *et al.,* 1984; Nedohula and Westhoff, 1997; Gelman *et al.*, 2001; Savvaidis *et al.,* 2002). The results of this study therefore agree with the findings of the above authors.

The recommended limits of TVC in fish by Kenya Bureau of Standards is 6 log cfu/g (KEBS, 2001). At the point of rejection the psychrophilic TVC for the ungutted experimental (Table 4.7) and control (Table 4.8) fish samples was 7.0 and 6.4 log cfu/g, respectively, while the mesophilic TVC was 6.7 and 6.3 log cfu/g, respectively. It can be explained that the levels of the mesophilic TVC were within the range of acceptability as recommended by ICSMF (1986) but not that of the psychrophilic TVC and therefore the psychrophilic TVC could form a basis for rejection of the fish samples.

The gutted, washed and iced experimental (Table 4.7) and control (Table 4.8) fish samples had significantly lower ($p<0.05$) levels of TVC and coliform counts than those of the corresponding ungutted experimental (Table 4.7) and control (Table 4.8) fish samples at any given sampling time. The levels increased significantly on the 14th and 18th day of storage in ice onwards, respectively. It may be explained that the TVC at any particular sampling time depended on the initial load in the fish flesh and level of hygienic handling. The gutting process may have reduced bacterial populations that are associated with the gut and reduced the number of bacteria that would penetrate the flesh of the fish hence resulting in lower bacterial counts (Papadopoulas *et al*., 2003). The washing of the fish may also have reduced the microbial load since the water used was treated tap water.

The coliforms are indicator bacteria that when present in any food signals the presence of enteric pathogens. They also show the hygienic conditions under which the food was produced and handled. The coliform organisms are found in the soil, plant materials and mud and can be dispersed into water bodies by surface runoff. Since the coliforms are both faecal and non-faecal, they are capable of multiplying outside the body. There was a significant increase (pÒ0.05) in the counts for coliform with storage time in ice for the ungutted experimental (Table 4.7) and control (Table 4.8) fish samples from the 14th day and 18th day of storage in ice onwards, respectively. The coliforms were able to breakdown the non protein components of the fish into volatile

amines which therefore raised the pH and therefore providing a favourable environment for multiplication of these organisms.

The recommended maximum acceptable limit for coliforms by the Kenya Bureau of Standards in fresh fish is 2 log cfu/g (KEBS, 2001). The presence of high counts of coliforms in water samples from the boats, shores and fishing ground and hence high counts in fish indicated poor hygienic practices and also the likely presence of *Escherichia coli*. This was confirmed by the presence of *E. coli* in the ungutted experimental fish samples and some control fish samples (Table 4.7 and 4.8). The presence of *E. coli* was an indication of faecal contamination brought about by poor hygienic practices. Its main sources could have been humans handling the fish, farm and wild animals, waterfowl, pets and the environment from which the fish was caught. It is important to note that the presence of *E. coli* may signal the presence of other potential human pathogens such as *Salmonella* spp., and *Shigella* spp. (Priscilla *et al.,* 2000). The presence of *E. coli* in these fish stresses the need for proper risk assessment and abatement procedures (good hygienic procedures and good handling procedures) to guarantee the safety of the fish and fish products that are supplied to the consumers (Satoshi *et al.,* 2007).

The absence of *E.coli* in gutted, washed and iced experimental and control fish samples indicated the high level of good hygienic practices observed during the gutting and washing process. The tap water that was used for the gutting process may also have eliminated the *E.coli* in the fish samples.

5.3.2 Hydrogen sulphide producing bacteria

Some bacteria such as *Shewanella putrifaciens* and *Vibrionaceae* are able to breakdown sulphur containing amino acid L-cysteine to produce H_2S. The H_2S which is a volatile sulphur compound has a very foul smell and therefore even a minimal quantity has considerable effect on quality. The H_2S producing bacteria counts for the ungutted experimental and control increased with increasing storage time. The increase in the H_2S producing bacterial counts also manifested itself in the changes in the sensory characteristics resulting in an increase in demerit points. The correlation between the H_2S producing bacterial counts and the quality index scores was high (r^2 = 0.98) (Fig. 4.4) indicating that the growth of these bacteria could have contributed to the observed sensory changes such as off-odour. This was also supported by the rejection of the cooked ungutted Nile perch experimental and control fish samples when a rotten odour with a

strong ammoniacal smell, a sour, fishy and musty taste, and a very soft and mealy texture was detected at the point of rejection on the 22nd and 28th day of storage in ice, respectively.

The gutted, washed and iced experimental (Table 4.7) and control (Table 4.8) fish samples showed H_2S producing bacteria counts that also increased significantly (pÒ0.05) from the 14th and 18th day of storage in ice onwards, respectively. However, the levels of these counts were significantly lower ($p<0.05$) compared to the levels for the ungutted fish samples at any sampling time (Table 4.7 and 4.8). The lower levels of H_2S producing bacteria could have contributed to the extension in the shelf life of the gutted fish samples and the lower QI scores compared to ungutted fish samples. The higher correlation of 0.98 (Table 4.11) between the H_2S producing bacteria and the QI scores supports the fact that these bacteria contributed considerably to the sensory changes that manifested throughout the storage period.

Salmonella spp. is one of the H_2S producing bacteria. These bacteria could not be detected in both the ungutted and gutted fish samples that were immediately iced after removal from the nets and those bought from the beaches. The absence of *Salmonella* spp. can be explained in a number of ways: including its poor resistance to cationic peptides in the fish flesh and also its ability to enter a viable but non-cultural state. The Nile perch fish species may have synthesized small cationic peptides that have antimicrobial properties against gram-negative and gram-positive bacteria and could have killed *Salmonella* spp. *Salmonella* spp. is known to enter into a viable but non-culturable state in which case their cells cannot be detected by standard culture on enriched media although they remain viable and capable of resuscitation under favourable conditions (Foster and Spector, 1995). However, this could not be confirmed in this study. This survival mechanism of *Salmonella* spp. can be a cause of public health concern. It is therefore important to state that the assurance of improved standards of hygiene along the landing beaches in Lake Victoria is of critical importance in ensuring safety of fish consumers.

5.4 Proximate composition

The lipid, ash, moisture contents of experimental and control (Table 4.9) fish samples were not significantly different (pÓ0.05) over the storage time in ice. Total crude protein decreased significantly (pÒ0.05) with storage time in the both ungutted gutted experimental and control fish samples from the 18th day of storage onwards. The decrease in the total crude protein content of ungutted fish flesh was possibly due to a decrease in salt soluble protein and water soluble protein (Chomnawang *et al,* 2007). This loss could also be due to autolytic deterioration

associated with the activity of endogenous enzymes and bacteria (Hultman and Rustad, 2004). This decrease coincided with an increase in the number of bacterial counts and this could be attributed to the proteolytic breakdown of the protein molecules to release volatile nitrogenous compounds determined as TVBN. (Table 4.7 and 4.8).

The gutted experimental and control (Table 4.9) fish samples had a slightly higher levels of protein at any sampling time compared to the ungutted experimental and control fish samples. These levels of protein were not significantly different (pÒ0.05) at any given sampling time. The slower rate of decrease in the crude protein content could be attributed to the gutting process which could have eliminated the endogenous enzymes responsible for proteolytic breakdown of protein molecules that result in the release of volatile nitrogenous compounds. The washing process may also have reduced the microbial loads which were responsible for the reduced breakdown of nitrogenous compounds compared to ungutted fish samples.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The findings of this study show that the selected sensory parameters were useful for developing Quality Index Method scheme for Nile perch over the storage time in ice. The initial parameters developed for the preliminary scheme included appearance of skin and slime, eyes clarity and shape, flesh color in open surfaces, adherence of the peritoneum, texture (flesh elasticity and belly texture), and the colour, odour of gills and gill slime. The maximum sum of demerit points for the preliminary scheme was 24. The final scheme consisted of 9 parameters covering different attributes, whose total sum was 20 demerit points at the worst condition of the stored fish. The scores for the selected quality attributes gave a linear relationship with a high correlation (R^2=0.98) with storage time in ice. This implied that the QIM scheme reflected the deteriorative changes in Nile perch with storage time accurately.

There was a general trend of an increase in the sensory, chemical and microbiological parameters studied with storage time in ice. However, the levels of these parameters tended to be lower for the gutted, washed and iced fish samples than for ungutted fish samples at any sampling time.

The observed changes in the sensory parameters used in the QIM scheme correlated well with changes in the chemical parameters (TVBN, FFA, and pH) and the microbiological parameters (TVC and H_2S producing bacteria counts) analyzed over the storage period. Hydrogen sulphide producing bacteria seemed to be the best parameter that determined the Quality index score with a high correlation of 0.98. This was followed by pH (0.97), TVBN (0.97), FFA (0.96) and TVC (0.93), respectively.

The selected sensory, microbiological and chemical parameters were appropriate for determining the shelf life of Nile perch. These parameters levels were close to, at or exceeded the maximum set levels by regulatory authorities or comparable to other levels found in other fishes during rejection. The maximum storage shelf life of the ungutted control Nile perch fish samples as determined with the descriptive sensory evaluation was 27-28 days in ice, while those that were gutted, washed and iced, had a longer shelf life of more than 28 days in ice. The ungutted experimental fish samples with a delay of 3-4 hours before icing, had a shelf life of 22 days, while the gutted fish samples from the same lots had a shelf life of 26 days. The quality index

scheme that was developed in this work can therefore be used to estimate the remaining storage time of the Nile perch in ice.

The nutritional composition of Nile perch showed that it has high content of protein of 19.5% when fresh which slightly decreased to 17.5% as the quality deteriorated. The fish is lean and had a low lipid content of 0.59-0.63%. The moisture and ash content varied between 78.5 and 79.6%, and 0.55 and 0.63%, respectively. The fish has a low fat content which is good for consumers requiring low fat diets.

6.1 RECOMMENDATIONS

The study has demonstrated that the Quality Index Method scheme developed can be a useful tool for assessing the freshness of the Lake Victoria Nile Perch and therefore the author recommends the following:-

I. The fishermen, the fish processors and traders should be encouraged to adopt the scheme since it is simple, cheap, reliable and relatively fast. The method requires no specialized equipment. It is far much better than relying on the laboratory tests which are expensive in terms of expertise and resources and take longer to establish the quality of fish under investigation.

II. The fishermen should be encouraged to practice icing immediately after removal of the fish from the net. This has been shown from this study to extend the shelf life of Nile perch by about four days for both the ungutted and gutted fish samples. The delay in icing should be discouraged as it reduces the shelf life of Nile perch.

III. The gutting, washing and icing has been shown from this study to increase the shelf life of Nile perch fish even after some delay in icing. The fishermen, fish traders and processors should be encouraged to practice the gutting and washing processes as they can contribute to a reduction in post harvest loss of this fish.

IV. The scheme should be adopted by the fishery sector to enable the setting of standards and guidelines for proper handling to retain prime quality of fish which will be necessary for access to new markets. Such standards and guidelines will be useful to the Fisheries Fish Inspectors and Kenya Bureau of Standards Inspectors in the enforcement of sensory quality of fish destined for the domestic and export markets, guidelines that are currently lacking. Occasional interruptions of exports to the European Union markets and the resultant huge financial losses to the industry will be minimized since standards and

guidelines for proper handling and enforcement of quality of fish will be set. This is bound to create steady employment to the locals and a steady growth of the sector and economy through increased export earnings.

V. The adoption of the method is likely to reduce post-harvest losses in the fisheries sector because fishermen, fish traders and fish processors will be able to establish the storage life and even further, predict the remaining storage life of their fish in ice. This is important because they will be able to know how long their fish will remain saleable. This will ensure that the fish remain of high commercial value, potentially improving their income, and improving food security in the sub-sector in line with the aspirations of the Millennium Development Goal No. 1 (eradicating absolute poverty) and the Economic Pillar for Economic Empowerment in Kenyaôs Vision 2030.

VI. Further research should be taken to improve the proposed Nile perch scheme by studying the influence of location, size, catching method and season on the suitability of the scheme.

VII. The scheme should be used as a guideline for developing QIM schemes for differently processed Nile perch fish products.

VIII. As a follow up to this study, there is a need to accurately determine the shelf life of the gutted Nile perch iced on boats immediately after removal from the nets.

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APPENDICES

Appendix 1
Evaluation of cooked fish

NAME DATE SPECIES

INSTRUCTION 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

WHOLE ☐
FISHFILLET ☐

é ..CODE

10	8	6	4	2	0				
Species specific Seaweed	Fresh fish, Slightly sour.	Slightly fishy, Sulphide	sour, stale, cabbage, Strong, ammonia	Rotten, spoilt putrid	Rotten,				

ODOUR

.....

Meat flavour, Sweet, watery, Initially no sweetness	Sweet and characteristic but reduces in intensity	Neutral, slightly fishy	Musty, fishy, slightly sour, some off-flavours	Slightly rotten, sulphide	Rotten, putrid

TASTE

.....

Firm, elastic, Flaky,	Firm,	Less firm,	Softer, Mealy	Very soft	Slippery, sloppy, greasy

TEXTURE

é é

10	8	6	4	2	0

Rejection level

OVERAL QUALITY SCORE ☐☐☐☐

Adapted from Huss, 1995.