



Microbiology and Spoilage Trail in Nile Perch (*Lates niloticus*), Lake Victoria, Tanzania



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Thesis of degree of MSc. Food Science
(Seafood processing)

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**Microbiology and Spoilage Trail in Nile perch
(*Lates niloticus*) Lake Victoria, Tanzania**

A thesis submitted for a partial fulfilment of the requirement for the degree of

Masters of Science in Food Science
(Seafood Processing)

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DECLARATION

I wish to declare that this work is sole done by me.
And was not done and submitted at any place before.

DEDICATION

*I sole dedicate output of this work to my beloved daughters (twins) and their mother
For accepting and missing my company through all of the study period*

ABSTRACT

The microbiological spoilage trail and storage time for iced whole Nile perch (*Lates niloticus*) and chilled fillets were studied. Similarly, microorganisms of surface lake water, bottom sediments and whole Nile perch from fishing grounds in Mwanza gulf was analysed in phase I. Phase II and III included iced whole Nile perch, laboratory and four establishments' chilled fillets, and all were stored at 0-3°C during shelf life study. Hence, the main goal of the study was to check prevalence of certain human pathogens, main spoilage organisms and establish shelf life (storage time) as well as effect of short and long storage of whole Nile perch in ice prior to processing into chilled fillets.

The results showed variable bacteria counts for sediments and Nile perch guts while lake water results were approximately below method detection limit 0-20cfu/ml. It was concluded that human pathogens occasionally may be derived from fishing grounds environments. The average keeping quality (shelf life) in days for iced whole Nile perch, laboratory and establishments chilled fillets were 26-28; 17; 8-13 days respectively. Shelf life was short (8 days) for Nile perch fillets produced from long time (17 days) stored whole Nile perch in ice before processing. In this work, the main spoilage organism's composition in spoiled whole Nile perch and chilled fillets may include *Shawenella putrefaciens*, *Vibrionaceae / Aeromonas*, *Pseudomonas*, and *Enterobacteriaceae*. For both sensory/organoleptic (QIM score) assessment and microbiological methods were consistent while chemical method was not reliable during early storages. Accordingly it was suggested that the chemical (TVB-N) parameter is not reliable indicator for freshness quality of iced whole Nile perch as well as chilled fillets.

Keywords: Nile perch, chilled fillets, specific spoilage organisms (SSO); spoilage micro flora, chemical (TVB-N) and storage days.

TABLE OF CONTENTS

DECLARATION	I
DEDICATION	II
ABSTRACT	III
1 INTRODUCTION.....	1
1.1 QUALITY AND SAFETY ASSURANCE OF EXPORTED FISH AND FISH PRODUCTS FROM TANZANIA.....	2
1.2 JUSTIFICATION OF THE STUDY	4
1.2.1 Objectives.....	5
2 BACK GROUND.....	7
2.1 MICROBIOLOGICAL SAFETY HAZARDS IN FISH INDUSTRY	7
2.2 RIGOR MORTIS AND POST HARVEST STORAGE OF FRESH FISH IN ICE	9
2.3 FRESH FISH SPOILAGE	9
2.3.1 Microbiological spoilage of fresh fish	10
2.3.2 Biochemical spoilage of fresh fish.....	14
2.4 STAGES OF SPOILAGE FOR ICED FRESH FISH	16
2.5 FACTORS CONTRIBUTING ON FISH SPOILAGE	18
2.5.1 Temperature	19
2.6 METHODS FOR ASSESSMENT OF FRESH FISH QUALITY	22
2.6.1 Sensory (qualitative) methods	22
2.6.2 Physical methods	23
2.6.3 Microbiological methods	24
2.6.4 Chemical methods	26
3 MATERIALS AND METHODS	28
3.1 STUDY AREA AND SAMPLING PLAN.....	28
3.2 SAMPLING.....	29
3.2.1 Phase I: Microbiology of Water, Sediments and Nile Perch.	29
3.2.2 Phase II: Shelf life for Laboratory iced whole Nile perch and chilled fillets	30
3.2.3 Phase III: Microbiology of whole Nile perch swabs, on line fillets and shelf life of establishments chilled fillets	31
3.3 PROCEDURES	32
3.3.1 Sensory (orgnoleptic) assessment	32
3.3.2 Microbiological analysis	33
3.3.3 Chemical analysis	39
3.3.4 Data analysis	39

4	RESULTS.....	40
4.1	PHASE I: MICROBIOLOGY OF WATER, SEDIMENTS AND WHOLE NILE PERCH.....	40
4.1.1	Phase I trial 1: Water.....	40
4.1.2	Phase I trial 2: Sediments.....	41
4.1.3	Phase I trial 3: Whole Nile perch.....	41
4.2	PHASE II: SHELF LIFE OF LABORATORY ICED WHOLE NILE PERCH AND CHILLED FILLETS.....	43
4.2.1	Phase II trial 1: Shelf life of iced whole Nile perch.....	43
4.2.2	Phase II trial 2: Shelf life of laboratory chilled Nile perch fillets.....	47
4.3	PHASE III: MICROBIOLOGY OF WHOLE NILE PERCH SWABS, ON LINE FILLETS AND SHELF LIFE OF ESTABLISHMENTS CHILLED FILLETS.....	49
4.3.1	Phase III - Trial 1: Microbiology of whole Nile perch swabs and online fillets.....	49
4.3.2	Phase III – Trial 2: Shelf life of establishments chilled fillets.....	51
4.4	PRESUMPTIVE SSOs.....	56
4.5	SHELF LIFE OF ICED WHOLE NILE PERCH AND CHILLED FILLETS.....	57
5	DISCUSSION.....	58
5.1	PHASE I: NATURAL MICROBIOLOGY OF WATER, SEDIMENTS AND WHOLE NILE PERCH.....	58
5.1.1	Phase I trial 1: Water.....	58
5.1.2	Phase I trial 2: Sediments.....	58
5.1.3	Phase I trial 3: Whole Nile perch.....	59
5.2	PHASE II: SHELF LIFE OF LABORATORY ICED WHOLE NILE PERCH AND CHILLED FILLETS.....	59
5.2.1	Phase II trial 1: Shelf life of iced whole Nile perch.....	59
5.2.2	Phase II trial 2: Shelf life of laboratory chilled fillets.....	62
5.3	PHASE III: MICROBIOLOGY OF WHOLE NILE PERCH SWABS, ON LINE FILLETS AND SHELF LIFE OF ESTABLISHMENTS CHILLED FILLETS.....	63
5.3.1	Phase III - Trial 1: Microbiology of whole Nile perch swabs and online fillets.....	63
5.3.2	Phase III – Trial 2: Shelf life of establishments chilled Nile perch fillets.....	65
5.4	PRESUMPTIVE SSO.....	68
6	CONCLUSIONS AND RECOMMENDATION.....	69
6.1	CONCLUSIONS.....	69
6.2	RECOMMENDATION.....	70
	ACKNOWLEDGEMENT.....	71
	REFERENCES.....	72
	APPENDICES.....	83

LIST OF FIGURES

FIGURE 1: REPARIAN DISTRICTS ALONG LAKE VICTORIA SHORE LINE (TANZANIA; UGANDA AND KENYA).....	1
FIGURE 2: SKETCH MAP OF THE SOUTH-EAST PART OF LAKE VICTORIA. FISHING GROUNDS OF THE VARIOUS LANDING SITES ARE INDICATED BY SHADING. S: SEMBA; I: IGOMBE AND K: KAYENZE, (FAO, 2009).....	28
FIGURE 3: 10 DAY'S WHOLE NILE PERCH (#1-3) FROM LEFT AND 33 DAYS WHOLE NILE PERCH (#4-SPOILED) RIGHT, RESPECTIVELY DURING STORAGE IN ICE AT LABORATORY CHILL ROOM (0-3 ⁰ C).	43
FIGURE 4: QIM SCORE FOR WHOLE NILE PERCH DURING STORAGE IN ICE.	44
FIGURE 5: MAIN CONTRIBUTING ATTRIBUTES TO QIM SCORE FOR WHOLE NILE PERCH DURING STORAGE IN ICE, (GA: GENERAL APPEARANCE).	44
FIGURE 6: CHANGES OF SPECIFIC SPOILAGE ORGANISM (SSO), TOTAL VIABLE COUNTS (TVC) AND ENTEROBACTERIACEAE (ENT.) ON	45
FIGURE 7: CHANGES OF SPECIFIC SPOILAGE ORGANISM (SSO), TOTAL VIABLE COUNTS (TVC), ENTEROBACTERIACEAE (ENT.) AND QIM SCORE IN WHOLE NILE PERCH DURING STORAGE IN ICE.	46
FIGURE 8: CHANGES OF SPECIFIC SPOILAGE ORGANISM (SSO), TOTAL VIABLE COUNTS (TVC), ENTEROBACTERIACEAE (ENT.) AND.....	46
FIGURE 9: CHANGES OF TVB-N IN WHOLE NILE PERCH DURING STORAGE IN ICE.....	47
FIGURE 10: CHANGES OF QIM AND TVBN IN WHOLE NILE PERCH DURING STORAGE IN ICE.....	47
FIGURE 11: CHANGES OF SPECIFIC SPOILAGE ORGANISM (SSO), TOTAL VIABLE COUNTS (TVC) AND ENTEROBACTERIACEAE (ENT.) IN CHILLED FILLETS FROM NEWLY CAUGHT NILE PERCH.	48
FIGURE 12: CHANGES OF TVB-N IN CHILLED FILLETS FROM NEWLY CAUGHT NILE PERCH.....	48
FIGURE 13: CHANGES OF SPECIFIC SPOILAGE ORGANISMS (SSO), TOTAL VIABLE COUNTS (TVC), ENTEROBACTERIACEAE (ENT.) AND TVB-N IN CHILLED FILLETS FROM NEWLY CAUGHT NILE PERCH.	49
FIGURE 14: WHOLE NILE PERCH IN STAINLESS CONTAINER AFTER WASHING AT ESTABLISHMENTS.	50
FIGURE 15: CHANGES OF SPECIFIC SPOILAGE ORGANISMS (SSO), TOTAL VIABLE COUNTS (TVC) AND ENTEROBACTERIACEAE (ENT.) ON CHILLED FILLETS IN E1.	52
FIGURE 16: CHANGES OF TVB-N ON CHILLED FILLETS IN E1	52
FIGURE 17: CHANGES OF SPECIFIC SPOILAGE ORGANISM (SSO), TOTAL VIABLE COUNTS (TVC), ENTEROBACTERIACEAE (ENT.) AND TVB-N ON CHILLED FILLETS.....	52
FIGURE 18: CHANGES OF SPECIFIC SPOILAGE ORGANISM (SSO), TOTAL VIABLE COUNTS (TVC) AND ENTEROBACTERIACEAE (ENT.) ON CHILLED FILLETS.	53
FIGURE 19: CHANGES OF TVB-N ON CHILLED FILLETS.....	53
FIGURE 20: CHANGES OF SPECIFIC SPOILAGE ORGANISM (SSO), TOTAL VIABLE COUNTS (TVC) AND ENTEROBACTERIACEAE (ENT.) AND TVB-N ON CHILLED FILLETS.	53
FIGURE 21: CHANGES OF SPECIFIC SPOILAGE ORGANISM (SSO), TOTAL VIABLE COUNTS (TVC) AND ENTEROBACTERIACEAE (ENT.) ON CHILLED FILLETS.....	54
FIGURE 22: CHANGES OF TVB-N ON CHILLED FILLETS.	54
FIGURE 23: CHANGES OF SPECIFIC SPOILAGE ORGANISMS (SSO) TOTAL VIABLE COUNTS, ENTEROBACTERIACEAE (ENT.) AND TVB ON CHILLED FILLETS.....	54
FIGURE 24: CHANGES OF SPECIFIC SPOILAGE ORGANISM (SSO), TOTAL VIABLE COUNTS (TVC) AND ENTEROBACTERIACEAE (ENT.) ON CHILLED FILLETS.....	55
FIGURE 25: CHANGES OF TVB-N ON CHILLED FILLETS.....	55
FIGURE 26: CHANGES OF SPECIFIC SPOILAGE ORGANISM (SSO), TOTAL VIABLE COUNTS (TVC), ENTEROBACTERIACEAE (ENT.) AND TVB-N ON CHILLED FILLETS.....	55
FIGURE 27: CHILLED FILLETS FROM ESTABLISHMENTS.....	56
FIGURE 28: FLOW CHART EMPLOYED FOR IDENTIFICATION OF GRAM-NEGATIVE SPECIFIC SPOILAGE BACTERIA IN SPOILING ICED WHOLE NILE PERCH AND CHILLED FILLETS STORED AT 0-3 ⁰ C.	57

LIST OF TABLES

TABLE 1: NILE PERCH EXPORT PROCESSING FLOW DIAGRAM FROM UP-STREAM TO IMPORTERS AND ASSOCIATED QUALITY CONTROL MEASURES.....	4
TABLE 2: RASFF NOTIFICATION: COUNTRY OF ORIGIN TANZANIA (EC, 2006).	7
TABLE 3: IDENTIFICATION TEST OF GRAM-NEGATIVE BACTERIA	13
TABLE 4: TYPICAL SPOILAGE COMPOUNDS DURING SPOILAGE OF FRESH FISH STORED AEROBICALLY PACKED IN ICE OR AT AMBIENT TEMPERATURE, (SOURCE: HUSS, H. 1995).	13
TABLE 5: STAGES OF SPOILAGE OF FRESH FISH STORED IN MELTING ICE (SOURCE: ICMFS, (1980B).....	17
TABLE 6: CARDINAL TEMPERATURE FOR MICROBIAL GROWTH (ADAMS AND MOSS, 2008).....	20
TABLE 7: GENERA THAT INCLUDE PSYCHROTROPHIC BACTERIA (ICMFS, 1980A)	20
TABLE 8: METHODS FOR DETERMINATION OF THE CONTENT OF BACTERIA IN SEAFOOD (HUSS, 1995).....	24
TABLE 9: COMMONLY USED AGAR MEDIA FOR ENUMERATION AND DETECTION OF BACTERIA ASSOCIATED WITH FISH SPOILAGE, (HOVDA, 2007).	26
TABLE 10: FRESHNESS RATINGS USING THE QUALITY ASSESSMENT SCHEME USED TO IDENTIFY THE QUALITY INDEX DEMERIT SCORE (LARSEN <i>ET AL.</i> 1992) MODIFIED.....	33
TABLE 12: MICROORGANISMS OF SURFACE LAKE WATER.....	40
TABLE 13: PATHOGENIC MICROORGANISMS OF SURFACE WATER.....	40
TABLE 14: MICROORGANISMS OF SEDIMENTS.....	41
TABLE 15: PATHOGENIC MICROORGANISMS OF SEDIMENTS.....	41
TABLE 16: PATHOGENIC MICROORGANISMS ON NILE PERCH SKIN.	42
TABLE 17: MICROORGANISMS OF NILE PERCH GUTS	42
TABLE 18: PATHOGENIC MICROORGANISMS OF NILE PERCH GUTS.....	42
TABLE 19: WHOLE NILE PERCH SWABS BEFORE AND AFTER WASH AT ESTABLISHMENTS.....	50
TABLE 20: ON LINE NILE PERCH FILLETS BEFORE AND AFTER WASH AT ESTABLISHMENTS. ...	51
TABLE 21: SHELF LIFE (ESTIMATED) AND SPOILAGE MICRO FLORA OF ICED WHOLE NILE PERCH AND CHILLED FILLETS STORED AT 0-3 ^o C.....	57

1 INTRODUCTION

Nile perch (*Lates niloticus*) which is a native to Ethiopia country was introduced in the Lake Victoria during late 1950s and also to other lakes in Africa. Lake Victoria, world's second largest fresh water body (second only to Lake Superior of North America in size) and the largest in the developing world, it occupies a surface area of 68,800km², and catchment area of 284,000 km². It has a shore line of approximately 3,500km long. The lake touches the Equator in its northern reaches and lies between latitude 0.7⁰ N - 3⁰ S and longitude 31.8⁰ E - 34.8⁰ E. It is a relatively shallow lake with an average depth of 40 metres and a maximum depth approximate 80 metres. The lake is shared by three reparian countries whereas Tanzania occupies about 49 percent of the lake, and, Uganda and Kenya share the remaining portion by 45 and 6 percent respectively (figure 1; Source FAO, 2007).



Figure 1: Reparian districts along Lake Victoria shore line (◇ Tanzania; △ Uganda and ☆ Kenya)

According to the Lake Victoria Fisheries Organization (LVFO, 2009), the Fisheries of Lake Victoria provide an immense source of income, employment, food and foreign exchange for

East Africa. The lake produces a fish catch of over 800,000 tonnes fish annually that currently leads to worth about US \$590 million of which US \$340 million is generated at the shore and a further US\$ 250 million a year is earned in exports from the Nile perch fishery. The lake fisheries employee's as direct or indirect, almost 2 million people with household incomes and meet the annual fish consumption needs of about 22 million people in the region. The fishery is supported by three main important fish stocks, the *Lates niloticus* (Nile perch), *Rastrineobola argentea* (Dagaa/Sardines) and *Oreochromis niloticus* (Nile Tilapia). Whereas over 75% of Nile perch is send directly to fish establishment for processing and export to overseas especially to the lucrative European Market, while dagaa and tilapia serve the local region and domestic markets. Nile perch contribute about 60% of the total catch from the lake fishery supplies of which over 80% of the total fish and fish products have been exported to abroad/overseas markets.

According to the Fisheries Division (FD) annual statistics report (2008). The total fish production in 2007 was 332,179.48mt whereas 98.68% (327,806.5mt) of production was from artisanal fisheries. Total revenues collection was about TZS 213 billion which generated TZS 7.5 billion as royalty. The fishery exports from Lake Victoria alone contributed about 92% of the total export where Nile perch fillets contributed 74% and other Nile perch products and dagaa 13%. Lake Tanganyika contributed about 4% while Marine fishery export contributed about 5%. These fisheries employ more than 160,000 direct fishers and about 4 million others in fisheries related activities. The overall contribution of fisheries sector to the economy of the country is 10% and to the Gross Domestic Production (GDP) about 3%.

1.1 QUALITY AND SAFETY ASSURANCE OF EXPORTED FISH AND FISH PRODUCTS FROM TANZANIA

Fish quality control standards and marketing section under Fisheries Division (FD) in the Ministry of Livestock Development and Fisheries is responsible for monitoring, surveillance, quality control and certifying fish and fishery products to meet national and international quality standards. This is enacted by the Fisheries Act No. 22 of 2003 and Fisheries Regulations of 2005. Thus in order to efficiently implement these roles, FD established a National Fish Quality Control Laboratory (NFQCL)-Nyegezi in 1997 at Lake

Victoria Zone in Mwanza City following the pressure from EU in respect to the export of Nile perch from Lake Victoria. Currently the laboratory verifies the effectiveness and efficiency of quality and safety assurance management systems in fish processing establishments, as well as monitoring chemical residues and contaminants in the environments associated to fish and fish products. The tests help the inspection services (regulatory body) to ensure enforcement of the Fisheries Act and its Regulations. The NFQCL-Nyegezi is currently accredited for microbiological analyses in fish (food and feeds) which include; total plate count, coliforms, *Enterobacteriaceae*, *Staphylococcus aureus*, *Salmonella*, and *Vibrio cholera*. The criteria/guidelines for fish and fish products are total plate count (1×10^5 cfu/g); coliforms (4×10^2 cfu/g), *Enterobacteriaceae* (1×10^3 cfu/g); *Staphylococcus aureus* (1×10^3 cfu/g); *Salmonella* (absent/25g), and *Vibrio cholerae* (absent/25g).

To enable all these FD have ensured that fish establishments have put in place quality management and safety assurance programmes in line to the Fisheries Regulations 2005. The programmes include; Good Manufacturing Practices (GMP), Good Hygiene Practices (GHP) and Hazards Analysis for Critical Control Points (HACCP) and post process verification by in-plant laboratories.

The Nile perch handling chain includes about six links namely, the fishermen, middlemen, agents, and or fish processing establishments, fish exporters, importers and retailers. Fishing is undertaken mainly by artisanal fishers by using canoes, which are either paddled or with an out-board power. Fishers normally use drift nets which are set overnight, and some use longlines with baits. The fish are landed at various identified landing sites for sale to traders (middlemen, agents or factories) who transport it to the factory or local retail markets. Only organoleptically (sensory) excellent and very good quality fish are selected for the factory processing. Usually fishers do not ice fish just after capture, and the catch is first iced by the buyers/traders after passing several (i.e. $\leq 4-6$) hours at landing sites. In accordance to the study by Karungi, Byaruhanga and Muyoga, (2004), there is little information available regarding the quality changes that occur in Nile perch as a result of delays in icing and how this affects storage stability of the Nile perch and its products when eventually chilled/frozen and stored at refrigeration/ frozen temperatures. The general processing flow diagram for Nile perch supply chain for export products from up- stream (fishing), establishments to importer is as shown in table 1.

Table 1: Nile perch export processing flow diagram from up-stream to importers and associated quality control measures.

1.	Fishing /fishers - No stringent quality and safety checks and icing of catch
2.	Transportation - Canoes/boats to landing sites without/with ice
3.	Landing sites - Physical and quality sensory checks and icing
4.	Transportation - Insulated trucks/boats with ice
5.	Fish establishment - Physical and Quality sensory checks
6.	Receiving/offloading - Swabs by establishments
7.	Washing whole fish – treated potable water- microbiological checks
8.	Filleting/gutting - Swabs from personnel and contact surfaces
9.	Skinning - Swabs from personnel and contact surfaces
10.	Trimming - Swabs from personnel and contact surfaces
11.	Shower washing – treated/ potable water
12.	Pre-packing –fillet samples
13.	Chilling/Freezing – Temperature checks/ fillet samples
14.	Packing - chilled fillets in styrofoam boxes and frozen in waxed cartons/ fillet samples
15.	Storage - Samples of finished chilled fillets for microbiological examination by competent authority (analyses for safety and quality/hygiene microorganisms)
16.	Dispatch/Export - Temperature, packaging checks and sensory checks/ supervision by Fish inspectors
17.	Importer receiving – Physical, microbiological quality and safety checks and feedback/ notification in case of RASFF.

1.2 JUSTIFICATION OF THE STUDY

According to Mossel *et al.*, (1995) the sole goal of microbiological control of food production is to supply safe, nutritious and palatable food with adequate shelf life at reasonable cost to consumers. The microbiological safety means the absence, exclusion or elimination of pathogenic organisms which is regarded different or separately from spoilage microorganisms. Therefore the key to microbiological control of both food safety and spoilage lies to the understanding and application of principals of microbial ecology which leads to the results of microbial growth, survival and or death. So far there are few studies on fresh water fish spoilage i.e. Lake Victoria Nile perch as well as on other tropical fresh water fishes compared to temperate water fishes. However, currently there are no analyses done for spoilage bacteria indicators, i.e. TVB-N, TMA, and regular laboratory sensory tests throughout the Nile perch handling chain.

1.2.1 Objectives

The purpose of study was to carry out trials for microbiological shelf life of freshly iced and hygienically handled Nile perch (in laboratory study) and normally factories (establishments) handled fillets as well as the prevalence of certain human pathogens. The study was divided into three phases. Phase I: Microbiology of lake water, sediments and whole Nile perch. Phase II: Shelf life of laboratory iced whole Nile perch and chilled fillets. Phase III: Shelf life study for normally processed chilled fillets at establishments.

1.2.1.1 Specific objectives

The specific objectives include:-

1. To check prevalence of certain human pathogens on whole Nile perch.
2. To examine microbiological spoilage indicator **trail** in iced whole Nile perch and chilled fillets.
3. To establish average storage time (shelf life) of iced whole Nile perch and chilled fillets.

1.2.1.2 Specific questions

The specific study questions include:-

1. Are the pathogenic contaminants in whole Nile perch derived from upstream (fishing ground or landing sites)?
2. Which are the dominant spoilage organisms in whole Nile perch and fillets from Lake Victoria?
3. What is the average keeping quality (shelf life) in days for iced fresh Nile perch, chilled fillets; and establishments processed chilled Nile perch fillets?
4. Is there significant difference in keeping quality between the chilled Nile perch fillets processed after (long and short time) storage of fresh whole Nile perch in ice?

1.2.1.3 Output from the study

The implementations of outputs from the study are expected to help:-

1. To strengthen and expand microbiological services offered by NFQCL-Nyegezi to the official fish inspection and the fish (food and feeds) industry sector.
2. To improve microbiological quality control and safety assurance measures, increase fish and fish products shelf life, and
3. To reduce post harvest losses and hence increase production and revenues.

2 BACK GROUND

2.1 MICROBIOLOGICAL SAFETY HAZARDS IN FISH INDUSTRY

In the recent years, the European Commission report (2006) lists some rapid alerts for food and feed (RASFF) notifications for Tanzania fish products exported to EU markets. These include about eight RASFF notifications from 2002 to 2006. Six of these RASFF notifications were on the presence of *Salmonella* species (Nile perch, in 2002 (2), in 2003 (1) and in 2004 (1); in frozen octopus, in 2005 (2)) and in 2006 on rupture of the cold chain (i.e., high temperature in chilled fillets to 11-17°C), see details table 2.

Table 2: RASFF notification: Country of origin Tanzania (EC, 2006).

REFERENC E	DATE	PRODUCT	SOURCE OF CONTAMINATION
2006/BTT	01/08/2006	Frozen Nile perch fillets (<i>Lates niloticus</i>)	Too high content of E 452- polyphosphates
2006/BJB	12/06/2006	Refrigerated Nile perch fillets (<i>Lates niloticus</i>)	Bad temperature control (rupture of the cold chain (+10 till +17 °C
2005/BCK	25/04/2005	Frozen octopus (<i>Octopus vulgaris</i>)	<i>Salmonella</i> spp. (presence /25g)
2005/AIT	11/02/2005	Frozen octopus (<i>Octopus vulgaris</i>)	<i>Salmonella</i> spp. (presence)
2004/CNS	23/11/2004	Chilled fillets of Nile perch(<i>Lates niloticus</i>)	<i>Salmonella</i> spp. (presence /25g)
2003/BLM	2/07/2003	Fresh chilled Nile perch	<i>Salmonella braenderup</i> (presence /25g) and <i>Salmonella leoben</i> (presence/25g)
2002/BDQ	24/09/2002	Nile perch (<i>Lates niloticus</i>)	<i>Salmonella</i> spp. (presence /25g)
2002/AQZ	14/06/2002	Nile perch fillets	<i>Salmonella</i> spp. (presence n=5, c=0)

Food is considered to be microbiologically unsafe owing to the presence of microorganisms which may invade human body (i.e. *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, etc) and also those that produce toxins ingested with a food such as *Staphylococcus aureus*, *Clostridium botulinum* and *Bacillus cereus*. The growth of these pathogens may not necessarily results in food spoilage. Hence the absence of deleterious sensory changes cannot be used as an indicator for food microbiological safety (Border and Norton, (1997). Consequently many outbreaks are caused by bacteria originating in the animal/human

reservoir i.e. *Salmonella*, *Shigella*, *E. coli*, and *Staphylococcus aureus*, (Huss, Dalgaard and Gram, 1997).

Generally fish and shellfish are common vehicles of transmitting food borne diseases. On the other hand, pathogenic bacteria (indigenous bacteria) represent part of the natural flora on particularly fish from coast and estuarine environment. According to Huss, Dalgaard and Gram, (1997), the indigenous (pathogenic) bacteria include *Vibrio spp.*, *C. botulinum* (Type E), *L. monocytogenes*, *Aeromonas spp.*, and *Shewanella alga*. However, the level of contamination is normally quite low and it is unlikely that the numbers which are naturally present in uncooked seafood are sufficient to cause disease in healthy human being. Hence fish as food has far better record on safety than the mammalian meat.

It is known that the primary source of cholera is feces of infected persons and transmission of bacteria with water and food. However, at present it has also been found that in certain areas the aquatic environment is a natural reservoir (habitat) for *Vibrio cholerae* 01. This means that seafood may be contaminated even if harvested in unpolluted waters, (Popovic *et al.*, (1993), Huss, Dalgaard and Gram, 1997). *Aeromonas spp.* that is pathogenic to both fish and human can be present in aquatic environment, on fish or even in tap (drinking) water (Gibson, 1992). The microbiologists have been taking the criteria to account that the presence of *Escherichia coli* in food indicates the probability of contamination of the food products with the sewage of human or animal origin. Consequently it has been referred as an indicator for the presence of pathogenic intestinal bacteria. On the other hand, certain *E. coli* strains (*EnteropathogenicEPEC*; *Enteroinvasive-EIEC*; *Enterotoxigenic-ETEC*; and *Enterohaemorrhagic-EHEC*) are pathogenic and lead to serious human diseases that can be transmitted with water and food (Doyle, 1990).

The faecal coliforms and *E. coli* are particularly useful as indicators of faecal contamination and poor handling of seafood's. This is because the organisms are absent from the fish at the time of capture except in grossly polluted waters. The aerobic plate counts (APC) or total viable count (TPC) is recommended for all fish and fish products because of its usefulness as an indicator of utility, the condition and length of storage of products prior preservation processes such as freezing. According to ICMSF, (1986), most aquatic animals at the time of harvest have counts in the region of $10^2 - 10^5$ organisms per gram. While an increase in APC/TVC to levels $>10^6$ per gram is an indicative of long storage at chill

temperatures or temperature abuse prior processing. APC is thus an indicative of quality and effectiveness of handling procedures and storage conditions.

2.2 RIGOR MORTIS AND POST HARVEST STORAGE OF FRESH FISH IN ICE

The live fish muscles are limp and pliable, but soon after death the muscles contract and become rigid, a state which is known to be in rigor. Rigor mortis refers to the processes that a fish undergoes immediately after death. This situation lasts for a period of time until the muscles relax and become soft again and the rigor is resolved. This phenomenon is important in fresh caught fish because the bacterial growth is retarded during the rigor period. Consequently rigor mortis in fish attributes to the quality and shelf life of processed fish products. However, the onset and duration of *rigor mortis* is dependent on temperature, the catching method used and the glycogen reserves of the fish. Amlacher (1961) suggested that the *rigor mortis* process determines the subsequent shelf life on ice since autolysis and bacterial decomposition could not start until after rigor resolved.

However, use of ice that is an ideal cooling medium for fresh fish. It rapidly cools fish and keeps it nearly at 0°C throughout the distribution from fishing to processing. It also continuously washes away bacteria, blood and slimes as it melts and prevents dehydration of fresh fish. In addition icing and chilling slow down bacterial and enzymic activities in fish. The preservation effect of icing can last for up to 2-3 weeks which depends on fish species (Santos-Yap, 1995). For example due to bruises and cuts caused by poor handling may lead to a nutritious environment for bacteria growth. The bruising may be reduced or avoided by not overfilling fresh fish in a storage containers and avoid throwing fish. Hence, during storage of newly caught fish, microorganisms may grow and result into softening and discoloring of some parts of flesh (muscles) of fresh fish. The rate for growth of microorganisms usually depends on the ability to tolerate the preservation conditions.

2.3 FRESH FISH SPOILAGE

Numerous works have been done in food products spoilage and still there is a need for more studies on various foods associated with complicated interactions causing spoilage.

Spoilage of food means to deprive it of its quality. In totality food spoilage is the results of metabolic processes that cause food to be undesirable for human consumption, (Adams and Moss, 2008; Doyle, 2007; Gram *et al.*, 2002, 1996). Therefore, generally spoilage is a subjective quality which means products may be accepted or rejected by one person while the same product may neither be accepted nor rejected by another person. Despite of the important role of microorganisms in food spoilage, the final decision for rejecting the spoiled food product concerned relies on sensory evaluation, (Gram *et al.*, 2002; Connell, 1975; and Reineccius, 1990). This is because the microbial spoilage is normally associated with a sudden onset which is noticed at late stages of spoilage of products when they (microbial growth) are at log phases or the exponential growth phases. Therefore, it is easier to identify chemical compounds that are responsible for a particular sensory index such as off odours than the responsible spoilage organisms.

According to Baird Parker, (2000) and Anonymous, (1985), the current world food post harvest and/or slaughter losses due to microbial spoilage estimates is about 25% of total food produced. This is a big loss which can be reduced if the ecology of specific spoilage organisms is well understood and controlled. However, every food product consistit of its own spoilage micro flora associated with the raw materials, or post contamination, effect of central processing parameters and storage conditions.

2.3.1 Microbiological spoilage of fresh fish

Despite that muscles of fresh or live fish are sterile, it contains high load of bacteria on the surface slime of the skin, on the gills and in the digestive tract. Bacterial loads on surfaces (skin) of fish from catch can range from hundreds up to millions per square centimetre ($10^2 - 10^7/\text{cm}^2$); and in the gills and intestines in the range of $10^3 - 10^9/\text{g}$ (Adams and Moss, 2008; Shewan, 1962; ICMSF, 1980b; Liston *et al.*, 1976). These bacteria include Gram-negatives of the genera *Pseudomonas*, *Shewanella*, *Psychrobacter*, *Vibrio*, *Flavobacterium*, and *Cytophaga* and some Gram-positives such as coryneforms and micrococci. When the fish is dead, the immune system collapses and bacteria are able to multiply. The bacteria on the skin surface to a large extent colonize the scale pockets and invade the flesh by moving between the muscle fibres during storage. Spoilage bacteria dominate and contaminate the flesh/muscles through damaged parts of flesh and cause rapid spoilage of fish. Murray and

Shewan (1979) reported that only a very limited number of bacteria invade the flesh during ice storage. The rate of spoilage is slow from the skin/surface in whole fish, increases for gutted fish followed by fillets and minced fish (ICMSF, 1980b).

Microbiological spoilage reactions in seafood depend on the initial composition or fish species, original environment and storage conditions, (Huss *et al.*, 1997, Gram *et al.*, 2002). Spoilage of fish is mainly due to the activity of psychrotrophic gram-negative bacteria such as *Shewanella putrefaciens* and *Pseudomonas spp.* *Shewanella putrefaciens* has been identified as the specific spoilage bacteria of marine temperate-water fish stored aerobically in ice. Some *Pseudomonas spp.* are the specific spoilers of iced stored tropical freshwater fish (Lima dos Santos, 1978; Gram *et al.*, 1990) and are also, together with *S. putrefaciens*, spoilers of marine tropical fish stored in ice (Gillespie and MacRae, 1975; Gram *et al.*, 1990). At ambient temperature, motile *Aeromonads* are the specific spoilers at aerobically stored freshwater fish (Gorzyka and Pek Poh Len, 1985; Gram *et al.*, 1990). Barile *et al.*, (1985) found that a large proportion of the flora at ambient-stored mackerel consisted of *S. putrefaciens*; this indicates that the bacterium may also take part in the spoilage of fish stored at ambient temperature.

The studies on ambient stored Nile perch by Gram L, and others (1990), found out the following composition of genera and species. The gram-negative *Aeromonas spp*, *Acinebacter*, *Alcaligenes* and *Moraxeralla*, and gram-positives included *Mircoccus* and *Staphylococci spp.* The bacterial counts when Nile perch was spoiled and rejected was up to level of $10^8/g$ in iron agar (IA) plates. The IA is used for enumerating hydrogen sulphite producing spoilage bacteria which forms black colonies in contrast to white colonies produced by other bacterial spp. *Pseudomonas spp.* dominated in spoiled Nile perch after 33 days stored in ice. The situation was the same with spoiled iced cod. Gram *et al.*, (1990); Lima dos Santos, (1978); and Cowel and Liston (1962) had all noted that the “bacterial flora on tropical fish resembles the micro flora on temperate fish species”. However, fish and fish products spoil by different specific spoilage organisms (SSO) depending on the final treatment or preservation and storage temperature. Examples of SSO in different fish and fish products include *Pseudomonas*, *Shewanella putrefaens*, *Photobactereum phosporeum*, *Aeromonas hydrophila*, and *Alteromonas putrefaciens*, *Vibrionaceae*, *Aeromodans*, *Moraxella*, *Acinetobacter*, *Enterobacteriaceae*; and Yeast and molds. Findings from some studies in fish and fish product on SSO include the following:-

- *Pseudomonas* and *Shewanella putrefaciens* are specific spoilage bacteria of iced fresh fish and fish products under refrigeration conditions (Gram and Huss 1996; Taoukis *et al.*, 1999).
- *Pseudomonas* which is a large and poorly defined group of microorganisms. The genus is in the group of aerobe Gram-negative, catalase and oxidase-positive rods. Many species have a psychrophilic nature and are regarded as part of the natural flora of fish (Hvda, 2007). The species can form aldehydes, ketones, esters and sulphides following food spoilage, causing odours described as fruity, rotten and sulphhydryl-like (Lund *et al.*, 2000).
- *Shewanella spp.* and *S. putrefaciens*: The bacterium *Shewanella* is a facultative anaerobe Gram-negative, oxidase- and catalase- positive rod in the *Shewanellaceae* family. *S. putrefaciens* is regarded as a specific spoilage bacterium of marine fish from temperate water, stored aerobically in ice (Gram *et al.*, 1987; Gram & Huss, 1996).
- Hozbor *et al.*, (2006), identified bacterial flora in iced sea salmon that consisted of *Pseudomonas spp.*, *S. putrefaciens*, and *Aeromonas* which dominated the spoilage trail as well as *Moraxella spp.*, *Acinetobacter* and lactose positive- *Enterobacteriaceae* and *Vibrios spp.* They are predominant spoilage species on chilled fresh fish under aerobic conditions, (Fonnesbech *et al.*, 2005; Hozbor *et al.*, 2006).
- *Aeromonas hydrophila*, a fermentative Gram –negative bacteria, produces characteristic spoilage off-flavour in fish stored in ice.
- *Alteromonas putrefaciens* (former *Pseudomonas putrefaciens*), non fermentative, H₂S producing organism was detected by using Peptone iron agar (PIA), (Levin, 1968) in chilled fish and fish products).
- Lactic acid bacteria (LAB) and *Photobacterium phosphoreum* grow in fish which is lightly salted, packed under CO₂ and stored refrigerated. Preservation by vacuum and CO₂ packing tends to inhibit respiratory microorganisms and selects for *Photobacterium phosphoreum* and *Lactic acid bacteria*, (Dalgaard, 2000).
- Preservation by organic acids favours growth of LAB and yeast, (Lyhs *et al.*, 2004). Yeast and molds grow in heavily wet-salted and dry salted fish respectively, Gram *et al.*, 1996)

Dainty, Shaw, Hardinger, and Michanie, (1979); and Gram *et al*, (1990), used the scheme in table 3 for the identification of Gram-negative bacteria or Gram-negative SSO in fish.

Table 3: Identification test of Gram-negative bacteria

	<i>S. putrefaciens</i>	<i>S. alga</i>	<i>Pseudomonas spp.</i>	<i>Vibrionance</i>	<i>Enterobacteriaceae</i>
Gram reaction	-	-	-	-	-
Shape	Rod	Rod	Rod	Rod	Rod
Motility	+	+	+	(+)	(+)
Oxidase	+	+	+	+	+
Catalase	+	+	+	+	+
Glucose	-/O	-/O	O	F	F
TMAO	+	+	(-)	+	+
H ₂ S	+	+	(-)	+	(+)
Ornithine - decarboxylase	+	+	(-)	+	±

In general different SSO are able to grow in different fish and fish products either in association or succession and produce different spoilage indicators or metabolites which include: see table 4 below.

Table 4: Typical spoilage compounds during spoilage of fresh fish stored aerobically packed in ice or at ambient temperature, (Source: Huss, H. 1995).

Specific spoilage organism -SSO		Typical spoilage compounds
<i>Shewanella putrefaciens</i>		TMA, H ₂ S, CH ₃ SH, (CH ₃) ₂ S, Hx
<i>Photobacterium phosphoreum</i>		TMA, Hx
<i>Pseudomonas spp.</i>		ketones, aldehydes, esters, non-H ₂ S sulphides
<i>Vibrionaceae spp.</i>		TMA, H ₂ S
<i>Anaerobic spoilers</i>		NH ₃ , acetic, butyric and propionic acid
Legend: Typical spoilage compounds and substrates		
Symbol	Compounds name	Substrates
TMA	Trimethylamine	Trimethylamine Oxide (TMAO)
H₂S	Hydrogen sulphide	Cysteine
CH₃SH	Methylmercaptan	Methionine
(CH₃)₂S	Dimethylsulphide	Methionine
Hx	Hypoxanthine	Inosine and IMP (inosine monophosphate)

NH₃	Ammonia	amino acids and urea
Acetate, CO ₂ (carbon dioxide), H ₂ O (Water)		carbohydrate and lactate
Esters, ketones, aldehydes		amino-acids (glycine, serine, leucine)

2.3.2 Biochemical spoilage of fresh fish

Fish is very perishable, high protein food which contains high level of amino acids. Total volatile basic amines (TVBA), a general term which includes trimethylamine, dimethylamine, ammonia and other total volatile basic nitrogenous (TVB-N) are compounds associated with seafood spoilage. The levels of TVB-N and TMAO are different from each fish species and even in different parts of the fish. The TVB-N consists of ammonia (NH₃), monomethylamine (MMA), dimethylamine (DMA), and trimethylamine (TMA). Their levels may change during bacterial activity and or enzymic degradation of trimethylamine oxide (TMAO) in fish. Marine fish and some freshwater fish contain trimethylamine oxide (TMAO) which is degraded by majority of spoilage microbes to trimethylamine (TMA). The SSOs utilizes the available TMAO in anaerobic respiration and produces off-odours and off-flavours following the formation of TMA, (Huss and Larsen, 1980; Gram *et al.*, 1987, 1990; Dalgaard *et al.*, 1993). Sulphur containing amino acids; cysteine and methionine decompose to liberate off-odours and off-flavours; hydrogen sulphides and methylmercaptane; aldehydes, ketones, esters, hypoxanthine as well as other low molecular weight compounds in spoiling fish, Herbert and Shewan, (1975, 1976); Ringo *et al.*, (1974) and Shewan, (1962).

Karnicki and Lima Dos Santos, (1985) reported estimates of TVB-N in Nile perch fillets stored for two days at ambient temperature in tightly closed bag to have initial value of 48.5 mg N/100g rising to 60.2 mg N/100g. TVB-N values for cod stored in ice are 35-40 mg N/100g, (Ozogul, 2000) and levels of 30-35 mg N/100g (Connell, 1995 and Huss, 1988) which have been considered the limits of acceptability for ice stored cold water fish. Therefore TVB-N values above these are regarded to indicate spoilage of the fish stored in ice.

Ammonia (NH₃) has been identified as a volatile component in a variety of spoiling fishes. It is formed by the bacterial degradation/ deamination of proteins, peptides and amino acids as

well as produced by the autolytic breakdown of adenosine monophosphate (AMP) in chilled seafood products. The level of ammonia in iced cod was not found to increase substantially until the sixteenth day of storage, (LeBlanc, 1987).

Generally there is no production of trimethylamine TMA during early stage of storage of fresh caught fish. At this stage bacteria obtain energy from an aerobic oxidation. The growth of the aerobic bacteria results in formation of anaerobic or microaerophilic niches in fish. Then at later stages SSO make use of TMAO as an electron acceptor by reducing the compound to TMA. The level of TMA in fresh rejected/spoiled fish by sensory assessment is within a level of 10-15 mg TMA-N/100g for aerobically stored fish and at the level of 30 mg TMA-N/100g in vacuum packed cod, (Dalgaard *et al.*, 1993).

The DMA component is generated by activities of the fish enzyme TMAO dimethylase (TMAO-ase), which converts TMAO into equimolar quantities of DMA and formaldehyde (FA). During frozen storage the DMA is produced by autolysis.

The biogenic amines are stable to thermal processing, and if they are found present in finished canned products, it is a good indication that the raw material was spoiled prior to heating. According to Huss, (1995), fish muscle has the ability to support the bacterial activity in production of a wide variety of amine compounds which result from the direct decarboxylation of amino-acids. Most spoilage bacteria that possess decarboxylase activity do so. Hence the organisms may cause raise of pH of the growth medium/substrate through the production of amines. Amines include histamine, putrescine, cadaverine and tyramine which are produced from the decarboxylation of histidine, ornithine, lysine and tyrosine, respectively. During fish spoilage the levels of each of the nucleotide catabolic intermediates rise and fall within the tissue.

Enzymes are involved in autolytic breakdown of adenosine triphosphate (ATP) to inosine monophosphate (IMP). On other hand, the spoilage bacteria activities convert the IMP to inosine (Ino) and then to hypoxanthine (Hx). Also, Hx has been shown to accumulate slowly in sterile fish tissue in parallel to development of TMA. Both Jorgensen *et al.*, (1988) and Dalgaard *et al.* (1993) showed a linear correlation between the contents of TMA and hypoxanthine during iced storage of packed cod.

According to Cornell, (1975), the primary oxidation products are lipid hydroperoxides. In the later stages of oxidation secondary oxidation products usually may be present and thus be indicative of a history of autoxidation. These products consist of aldehydes, ketones, short chain fatty acids and others, many of which are characterised by unpleasant odours and flavours associated with oxidized fish lipids.

2.4 STAGES OF SPOILAGE FOR ICED FRESH FISH

The spoilage starts as soon as the fish dies due to series of chemical, physical, bacteriological, and histological changes that occur in the muscle tissue, (Emilia and Santos, 1996). When fish is being caught it uses a lot of energy from limited glycogen compound in the course of struggle to survive. The defensive power is reduced following depletion of glycogen in the muscles of fish. Depletion of glycogen limits the degree of post mortem acidification of the tissue; hence the pH remains between 6.2-6.5 as compared to around pH 5.5 for red meat. The pH is generally reduced due to the formation of lactic acid from glycogen which plays a role on retarding the growth of bacteria like in the case of the red animal meat. Therefore majority of fish spoil fast due to the favourable pH which supports the activity and growth of microorganisms. However prolonged keeping quality has been observed to fish which have low pH such as halibut which has approximately pH 5.6, (Adams and Mourice (2008). On the other hand, fish like halibut which contains natural fats in the form of phospholipids and is rich in trimethylamine oxide (TMAO) can also be more vulnerable to fast spoilage compared to lean fish. The fish-fat is usually highly unsaturated and it is easily oxidized (Santos-Yap, 1995). The rapid deterioration or spoilage of fresh fish as opposed to other high protein muscle foods is due to the large concentration of non protein nitrogen (NPN) compounds in fish muscles. Usually there are about four stages of fish spoilage as categorized by the number of storage days in melting ice. Starting from; 0-5 days, 5-10 days, 10-14, and after 14 days, in the following table 5.

Table 5: Stages of spoilage of fresh fish stored in melting ice (Source: ICMSF, (1980b).

Stages	Days In ice	Physicochemical reactions (<i>substrate & spoilage compounds</i>)	Microbiological (<i>SSO</i>) growth & Bacteria counts	Organoleptic/sensory changes	Chemical changes
I	0-5	Flesh undergoes rigor mortis ATP is converted to inosine	The dominant bacteria change $10^2 - 10^3/cm^2$	Eyes bright; Flesh firm Colour good; Gills bright Odour fresh	TMA \leq 1-1.5mg% VRS \leq 2-8units
II	5-10	Inosine is converted to hypoxanthine level of NH_3 increases Trimethylamine oxide (TMAO) is converted to trimethyleamine (TMA)	Bacteria growth occurs $10^3 - 10^6/cm^2$	Eyes begin to dull; Colour good; Gills colour fades; Skin colour fades Odour natural - slightly fishy Texture softens	TMA < 5mg% VRS Ca 5-10units TVB \leq 15mg%
III	10-14	Hypoxanthine is converted to xantine and uric acid; etc. TMA, total volatile bases (TVB) and total volatile acids (TVA) increase	Rapid bacterial growth occurs $10^6 - 10^8/cm^2$	Eyes sunken Gills discoloured and slimy Skin bleached Odour sour and fishy Texture soft	TMA < 10mg% VRS Ca 10-15units TVB 20-30mg% TBA 15-20mg%
IV	>14	Proteolysis TVA and TVB levels increase rapidly H_2S is produced Physical deterioration occurs	H_2S is produced by SSO (bacteria) $\sim 10^8/cm^2$ Bacteria number constant	Eyes opaque and sunken Gills bleached and slimy Texture very soft Odour offensive	TMA > 10mg% VRS CA > 20units TVB > 30mg% TVA > 60mg% H_2S , indole etc detected

Legend: mg% = mgN/100g; i.e. TMA: Trimethylamine expressed as mg/100g fish; TVB: Total volatile base expressed as mg/100g fish; TVA: Total volatile acids expressed as mg/100g fish; VRS: Volatile reducing substances expressed as mg/100g fish.

The gram-negative, rod shaped bacteria dominate the growth in chill temperature stored fish, (Liston, 1978). During an aerobic iced storage of fish, after 1-2 weeks, the spoilage flora may consist almost exclusively of *Pseudomonas spp.* and *S. putrefaciens*. This is due to their relatively short generation time at chill temperatures (Morita, 1975; Devaraju and Setty, 1985). These bacteria are able to grow at low temperature ($< 5^0C$) by utilizing the NPN compounds which are mainly available substrates in fish at early stage of storage in ice. The main spoilage bacteria at this stage are frequently *Pseudomonas spp.* Oxidative deamination follows, and leads to production of volatile amines dominated by ammonia (NH_3) and free fatty acids. Then reducing bacteria dominate by reducing the TMAO to TMA compounds. Huss *et al.*, (1988) found that TMAO reduction to TMA started when the level of specific spoilage bacteria, hydrogen sulphite producers like *S. putrefaciens* (black colonies) reached 10^7 cfu/ml in fish slurry and Gram *et al.*, (1990) 10^8 cfu/g in Nile perch (tropical fresh water fish) respectively when stored in ice. TMA is a major component which is associated with unacceptable odour of spoiling fish. The total volatile base (TVB) and total volatile acid (TVA) components produced during degradation of proteins are used

as well as indices of fish spoilage. Spoilage is also recognized when there is a significant accumulation of sulphur-containing compounds such as methyl mercaptan, dimethyl sulphide and H₂S produced by *Pseudomonas putrefaciens*. In 1970 Walker P., Cann D., and Shewan J. M., reported the bacteria spoilage association in the order of: the dominant flora at the first stage included mixture of *Acinetobacter-Moraxella* and *Pseudomonas*; then *Acinetobacter-Moraxella*, *Flavobacteria* and *Pseudomonas* and finally during the exponential phase the *Pseudomonas* became the dominant genus with partly *Acinetobacter* and *Moraxella*.

Studies by Gram *et al.*, (1986); Ravn Jorgensen *et al.*, (1988); and Gram, (1990) in temperate and warm fish indicated significance difference in keeping qualities in ice. Temperate water-fish and fish products when stored in melting ice spoiled after 9-10days and seldom after 2-3 weeks (14-21days), while tropical water fishes generally spoiled after 4 weeks (28 days) of ice storage (Nile perch). According to Gram *et al.*, (1986,1990) temperate water fishes when stored at ambient temperature were rejected after one day and warm water fish - Nile perch stored at 20⁰C- 30⁰C spoiled after 11-17 hours,. Fresh fish caught from warm tropical waters stored in melting ice was found to cause decrease in bacterial counts and extend the bacterial lag phase from 5 to 7 days before exponential phase started. However in the fourth week of storage at the point of rejection the total counts usually reach 10⁶ - 10⁸ cfu/g; of which 10⁶ – 10⁷ cfu/g represent counts for H₂S producing bacteria on spoiled and rejected Nile perch, (Gram *et al.*, 1990).

2.5 FACTORS CONTRIBUTING ON FISH SPOILAGE

Factors contributing to spoilage of food (fish) include insect's infestations and rodents, parasites; and activities of bacteria, yeast and molds in food that may render food to become undesirable. Exposure of food products to the light may cause degradation of proteins and fats to produce off flavours and off odours, (Doyle, 2007, Gram *et al.*, 2002). According to Huss, (1995) review, the storage or shelf life of fish and fish products may be influenced by fish species, fishing grounds, fishing season, pH and hygienic handling. Large, lean, thick skin, high pH, and flat fish species keep longer than small, fatty, thin skin, low pH and round fish species. Bruising/damaging fish during handling causes rapid spoilage. The wide range of micro flora in fish is reflected by its environmental habitats

such as fresh and salt waters; tropical and arctic waters, pelagic and bottom fisheries, degree of pollution of waters and degree of processing or preservation like icing, freezing, canning, and atmosphere/vacuum packing (Gram *et al.*, 1996). Hence the micro flora growing in fish products is determined by intrinsic and extrinsic factors like post mortem pH > 6.0, amount of non-protein-nitrogen (NPN) / or presence of TMAO.

The parameters which may be responsible for microbial spoilage in foods may be categorized into four groups (Adams and Moss, 2008): Extrinsic parameters, intrinsic parameters, central processing parameters and implicit parameters. Intrinsic parameters include physical, chemical, and structural properties of the food itself, such as water activity, pH, redox potential (E^h), available nutrients, and natural antimicrobial substances. Extrinsic parameters or environmental factors include storage time, temperature, humidity, and the composition of the storage atmosphere. Modes of processing and preservation treatments may change the characteristics of the food product, and microorganisms associated with the product. Implicit parameters or mutual factors antagonistically or in association influence microbial growth. The growth of one bacterial sub-population may affect other sub-populations in a food product.

2.5.1 Temperature

Temperature is the main environmental factor that affects growth and viability of microorganisms. Despite that, the range of temperature which permits growth of specific organisms, seldom exceeds 35°C, the microbial growth can occur at temperatures from about -8°C to +90°C (Adams and Moss, 2008). The microbial growth rate becomes stable at their optimal growth of about 20 to 30°C for psychrotrophs and 35 to 45°C for mesophiles, (ICMSF, 1980a). Consequently inhibition of their growth occurs at temperatures only slightly above the maximum optimal growth. However, there are four physiological groups of bacteria which are defined and distinguished by their temperature ranges of growth in (table 6): thermophiles, mesophiles, psychrophiles and psychrotrophs.

Table 6: Cardinal temperature for microbial growth (Adams and Moss, 2008).

Bacteria group	Minimum	Optimum	Maximum
Thermophile	40 - 45°C	55 -75°C	60 - 90°C
Mesophiles	5 - 15°C	30 - 45°C	35 - 47°C
Psychrophiles	-5- +5 ⁰ C	12 - 15°C	15 - 20°C
Psychrotrophs	-5- +5 ⁰ C	25 - 30 ⁰ C	30- 35 ⁰ C

(Adapted in ICMF, 1980: Cardinal temperature for Prokaryotic Microorganisms)

Mesophiles include human or animal pathogens and food spoilage bacteria. In favourable medium and at optimum growth temperature their generation time is ≤ 0.5 hr. The psychrophiles include all organisms that are capable to grow at 0°C. The psychrotrophs are found at wider range of temperatures and in more diverse range of habitats and thus they are of greater importance in the spoilage of chilled foods. The psychrotrophs include gram-negative and gram- positive bacteria; aerobes, anaerobes, and facultative anaerobes, motile and non-motile organisms; spore formers and non-spore formers. Some psychrotrophs species among them at least 27 genera are listed in table 7. Yeast includes psychrotrophic strains in the genera *Candida*, *Torulopsis*, *Cryptococcus* and *Rhodotorola*. Psychrotrophic molds are found in the genera *Penicillium*, *Trichothecium* and *Aspergillus*.

Table 7: Genera that include psychrotrophic bacteria (ICMFS, 1980a)

<i>Acinetobacter</i>	<i>Aeromonas</i>	<i>Alkaligens</i>	<i>Athrobacter</i>
<i>Bacillus</i>	<i>Chromobacterium</i>	<i>Cinetobacter</i>	<i>Clostridium</i>
<i>Corynebacterium</i>	<i>Enterobacter</i>	<i>Erwinia</i>	<i>Escherichia</i>
<i>Flavobacterium</i>	<i>Klebsiella</i>	<i>Lactobacillus</i>	<i>Leuconostoc</i>
<i>Listeria</i>	<i>Microbacterium</i>	<i>Micrococcus</i>	<i>Moraxella</i>
<i>Proteus</i>	<i>Pseudomonas</i>	<i>Serratia</i>	<i>Streptococcus</i>
<i>Streptomyces</i>	<i>Vibrio</i>	<i>Yersinia</i>	

2.5.1.1 Effect of storage at ambient and refrigeration temperature (0-25°C)

The storage temperature of world fresh fishery/seafood products is in the range within 0-25°C, (Huss, 1995). In this range of temperature the microbiological activities are important in the shelf life and spoilage of fresh fish stored at chill conditions in melting ice approaching 0°C and at ambient temperature; warm/tropical countries from between 10–30°C. However at temperature below 10°C mesophilic bacteria do not grow and

psychrotrophic organism grow at very slow rates with extended lag phases when the temperature is approaching at 0°C.

The psychrotrophic gram-negative rod shaped bacteria belonging to genera *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Shewanella*, *Flavobacterium*, *Vibrio*, *Aeromonas* and *Micrococcus* dominate micro flora in temperate waters. While the Gram-positive bacteria such as *Bacillus*, *Micrococcus*, *Clostridium*, *Lactobacillus* and *Corynebacterium* are found to dominate in higher temperature waters in varying proportions (Liston, 1980 & 1992; Hozbor, 2006; Gram *et al.*, 1990). Fresh water fish's micro flora is dominated by the *Aeromonas* spp. The micro flora consisting of *Pseudomonas*, *Acinetobacter*, *Moraxella* and *Vibrionaceae* was reported in newly caught fish in tropical Indian marine waters studies (Surendran *et al.*, 1989 and Huss, 1995). Findings from studies suggested that fresh water fishes have micro flora loads similar to the temperate water fishes with slightly higher in Gram-positive and enteric bacteria (Liston, 1980; Gram *et al.*, 1990).

At 0°C the microorganism's growth rate is less than one-tenth of the rate at the optimal growth rates. The relative rate of spoilage of tropical fish species stored at 20-30°C is approximates 25 times higher than at 0°C (Gibson and Ogden, 1987; Dalgaard and Huss, 1994). At 0-5°C *Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Aeromonas* spp. and *Pseudomonas* spp. are the dominant spoilage micro flora in iced stored fish. When fish is stored in ice the flora slowly doubles in one day (24hours) and after 2-3 weeks they reach numbers of 10^8 – 10^9 cfu/cm²/g. Tropical waters fishes first pass their lag phase after 1-2 weeks when stored in ice, (Gram *et al.*, 1990; Gram 1990). On the other hand at 15-30°C *Vibrionaceae* spp. and *Enterobacteriaceae* spp. are the dominant spoilage micro flora in fish, (Gram *et al.*, 1987, Gram *et al.*, 1990, Liston, 1992). At these ambient temperatures, the micro flora may grow up to counts of 10^7 – 10^8 cfu/cm²/g within one day (24hrs).

2.5.1.2 Effect of storage at super chilling temperature at 0°C to -4°C

The extension of shelf life of chilled fish fillets is in high demand in line with the transportation of fresh products to distant markets at lower cost (Olafsdottir *et al.* 2005). Superchilling has proven to effectively extend bacterial growth lag phase and prolong the shelf life of chilled fish and fish products (Huss 1995, Chang *et al.* 1998). Super chilling/partial freezing at 0°C to -4°C, has been used in seafood products when ice storage

is insufficient for good quality and extension of their shelf life. At present super chilling of fresh chilled fish products is applied by some fish establishments. Fish fillets/products are rapidly chilled to below 0 to -2°C by using either blast, plate or tunnel freezers. Various types of cooling systems have been used for super chilling (at -4 to 0°C) of seafood products. Examples of the cooling systems include chilled and refrigerated seawater (Smith *et al.* 1980, Olafsdottir *et al.* 2000), liquid-ice and brine solutions (Huidobro *et al.* 2002), flake ice or slurry ice (Losada *et al.* 2005, Zeng *et al.* 2005), subzero storage temperature; -2 and -3°C, (Riaz-Fatima *et al.* 1988, Sivertsvik *et al.* 2003) and the use of cooling agents like CO₂ and snow (LeBlanc and LeBlanc 1992, Jeyasekaran *et al.* 2004).

2.6 METHODS FOR ASSESSMENT OF FRESH FISH QUALITY

The qualitative and quantitative detection methods for SSO are in place. They include physical, sensory, microbial and biochemical (chemical) determination methods.

2.6.1 Sensory (qualitative) methods

Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyze and interpret reactions to quality attributes of food as perceived through the human senses. The most used sensory assessment of fish freshness employs trained persons called panellist to assess the fish by using the five common senses of humans, by seeing (eye), touching (skin), smelling (nose), hearing (ear) and taste (tongue) against a pre-set ranking criteria. EU scheme and Quality Index Method (QIM) have been the most promising methods in measuring the freshness of whole fish, which are rapid and reliable. Instruments as well may be used to measure some sensory profiles (parameters) i.e. Instron, Bohlin Rheometer for measuring texture and other rheological properties; microscopic methods combined with image analyser are used to assess structural changes and "the artificial nose" to evaluate odour profile (Nanto *et al.*, 1993). The sensory evaluation is generally the satisfactory and important method for assessment of fish freshness and or spoilage, (Connell, 1975; Reineccius, 1990). It has been commonly used in the fish sector and fish inspection services (Luten and Martinsdóttir 1997).

The Quality Index Method (QIM) that was developed by the Tasmanian Food Research unit (CSIRO), (Bremner *et al.*, 1985) has been used in the Nordic and Europe countries. QIM was primarily used for the evaluation of whole and gutted fish. Up to now, QIM schemes have been developed for a number of fish species including: fresh herring (Larsen *et al.*, 1992), cod (Jonsdottir 1992), red fish (Martinsdottir and Arnason 1992), Atlantic mackerel, horse mackerel and European sardine (Andrade *et al.* 1997), brill, dab, haddock, pollock, sole, turbot, shrimp and gilthead seabream (Huidobro *et al.* 2000), frozen cod fillets (Warm *et al.* 1998), farmed Atlantic salmon (Sveinsdottir *et al.* 2003) and fresh cod fillets (Bonilla *et al.* 2006). The application of QIM is excellent in the first part of the storage period i.e. fish stored in ice. During first stage of storage other instrumental methods results are inaccurate and or not reliable (Nielsen *et al.* 1992).

The QIM technique is based on selecting a number of significant sensory parameters (skin, eyes, gills, etc) or characteristic for a particular species and allocating scores to each attribute depending on the state of freshness or quality of the selected fishery products (Martinsdóttir 2002, Sveinsdóttir *et al.* 2003). QIM score grades from zero being the highest score (0) which is given for very fresh fish and increasing higher scores for the fish which is deteriorating, the grading scores are (0, 1, 2, & 3). The aim of an ultimate result is to be able to predict the remaining storage life of fish stored in ice (Larsen *et al.* 1992, Nielsen and Jessen 1997, Hydilg and Nielsen 1998, Martinsdóttir *et al.* 2001). It is suggested that, when the sum of score of a batch of fish reach demerit points of 10, the remaining keeping time in ice may be estimated to about five days, (Huss, 1995). Example of freshness ratings using the quality assessment scheme used to identify the quality index demerit score developed by Larsen *et al.* (1992) is given in appendix 1.

2.6.2 Physical methods

According to Huss, (1995), there are some trials that have been done in developing physical methods for freshness assessment of fish through measuring the micro-structural characterization of the fish muscle or texture, changes in electrical properties during rigor mortis stages, changes in colour, *pH*, and *Eh*. The electrical properties of fish skin and tissue changes after death are definitely compelling/demanding the need for physical measurements during the fish postmortem changes or spoilage. However, the wide differences in intrinsic and extrinsic factors, and heterogeneity characteristics of fish species, are the major factors

which are posing difficulties in developing physical measurements. Jason and Richards, (1975) came up with instrument, GR Torrymeter which can only grade batches of fish and is not able to measure quality or freshness of a single fish. The pH is the only measurement which has been commonly used as physical method for quality assessment by using pH-meter directly into the fish muscle or in suspension of fish flesh/muscles in distilled water.

2.6.3 Microbiological methods

The microbiological analysis is the second most used traditional method which determines the quantity of microbial cells in particular foods referred to as total counts. Traditional methods for bacteriological examinations can be laborious, time-consuming, and costly compared to sensory and chemical methods which only take few hours, (Huss, 1995). Common traditional plate count agars (PCA) are still the substrates widely used for determination of total counts. Total count represents the total number of bacteria that are capable of forming visible colonies on a culture media at a given temperature. An example of different methods used for determination of bacterial counts in fish and fish products are given in table 8 below.

Table 8: Methods for determination of the content of bacteria in seafood (Huss, 1995)

Method	Temperature, °C	Incubation cfu/g	Sensitivity
Plate count or Iron agar	15-25	3-5 days	10
"Redigel"/"Petrifilm(tm) SM"	15-25	3-5 days	10
Microcolony-DEFT	15-30	3-4 hours	10 ⁴ -10 ⁵
DEFT	--	30 min.	10 ⁴ -10 ⁵
ATP	--	1 hour	10 ⁴ -10 ⁵
Limulus lysate test	--	2-3 hours	10 ³ -10 ⁴
Microcalorimetry/ Dye reduction/ Conductance/Capacitance	15-25	4-40 hours	10

When examining bacteria in seafoods, more nutrient rich agar (Iron Agar, Lyngby, and Oxoid) gives significantly higher counts than PCA (Gram, 1990). Iron agar yields both the number of specific spoilage (hydrogen sulphide producing) and non spoilage bacteria. The incubation temperature dictates in the total counts. Incubation at $\geq 30^{\circ}\text{C}$ seems to be inappropriate when examining seafood products being held at chill temperatures. Pour

plating for 3-4 days and incubation at 25°C for psychrotrophs is recommended by surface plating and incubation at 15°C to avoid killing heat sensitive bacteria, (Huss, 1995). Ready made plates are available for easy handling such as "Redigel"/"Petriefilm(tm) SM", (Fung *et al.*, 1987). The *Redigel* (RCR Scientific) and *Petriefilm*TM SM (3M Company) are dried agars with a gelling agent to which the samples are directly added.

Direct microscopic count/ microcolony-DEFT: Microscopic examination of foods is a rapid way of estimating bacterial levels. By phase contrast microscopy the level of bacteria in a sample can be determined within one log-unit. One cell per field of vision equals approximately 5-10⁵cfu/ml at 1000 X magnification. Whilst microscopic methods are very rapid, their low sensitivity is considered as their major drawbacks. With the *ATP measurement & Limulus lysate test*, the bacterial numbers in foods are estimated by measuring the amount of bacterial adenosine triphosphate (ATP), (Sharpe *et al.*, 1970) or by measuring the amount of endotoxin (Gram-negative bacteria) by the Limulus amoebocytes lysate (LAL) test, (Gram, 1992). The former is very rapid but difficulties exist in separating bacterial and somatic ATP.

Microcalorimetry, dye reduction, conductance and capacitance methods are used for rapid estimation of bacterial numbers, based on the withdrawn sample, incubation at high temperature (20-25°C) and detection for a signal. The detection time (DT) is inversely related to the initial number of bacteria, i.e., early reaction indicates a high bacteria count in the sample and reversely proportional to the bacterial count obtained in agar methods.

2.6.3.1 Detection of specific spoilage organism (SSO)

Currently there are different peptone-rich (i.e. IA) substrates containing ferric citrate or sulphur-containing amino acids which have been employed for detection of H₂S-producing (SSO) bacteria such as *Shewanella putrefaciens* that forms black colonies due to precipitation of FeS (Levin, 1968; Gram *et al.*, 1987). Similarly *Vibrionaceae* forms black colonies on an iron agar to which an organic sulphur source is added (e.g., Iron Agar, Lyngby). The use of IA has been described by Van Spreekens, (1974); Gram *et al.*, (1987); Jensen and Schulz, (1980). Gram *et al.*, 1987, and reported that detection medium which contains only sulphur is not sufficient to detect all spoilage organisms such as *Aeromonas/*

Vibrionaceae at higher temperatures. Also there is still no selective or indicative medium available for the *Pseudomonas spp.* which spoil some tropical and fresh water fish or for *Photobacterium phosphoreum* that spoil packed fresh fish. However there are some common used media in enumeration of some spoilage bacteria shown in table 9 below.

Table 9: Commonly used agar media for enumeration and detection of bacteria associated with fish spoilage, (Hovda, 2007).

Bacterium	Medium	Reference
<i>Photobacterium phosphoreum</i>	Plate Count Agar, (PCA), modified by addition of 1 % NaCl	Nordic Committee on Food Analysis, 2000
	Long and Hammer	Van Spreekens, 1974
<i>Shewanella putrefaciens</i>	Iron agar (<i>black colonies</i>)	Gram <i>et al.</i> , 1987
Aerobic plate count	Iron agar (<i>black + white colonies</i>)	Gram <i>et al.</i> , 1987
	Long and Hammer	Van Spreekens, 1974
<i>Pseudomonas</i>	<i>Pseudomonas</i> agar base, (PAB)	Mead & Adams, 1977
<i>Brochothrix thermosphacta</i>	Streptomycin thallos acetate actidione agar, (STAA)	Gardner, 1966
<i>Lactic acid bacteria</i>	Man-Rogosa-Sharke, (MRS)	Baird <i>et al.</i> , 1987

2.6.4 Chemical methods

Besides sensory, microbiological and physical methods, also chemical analyses have been used to assess the freshness quality of fish (Gill, 1992). But like for the sensory methods, also for the chemical quantified analyses must also relay on increases or decreases of the levels of microbial spoilage or autolysis. This is because some indicator chemical compounds are not present/detected until the product is close to spoilage or after passing several days of storage of fresh fish in ice until the microbial counts become $>10^7$ cfu/g (Gram *et al.* 2002; Adams and Moss, 2008).

Total volatile basic amine (TVBA) is one of the most widely used measurements of seafood quality. It is a general term which includes the measurement of TMA (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile basic nitrogenous compounds associated with seafood spoilage. The TVBA measurements reflect only later stages of advanced spoilage and are generally considered unreliable for the

measurement of spoilage during the first ten days of chilled storage of cod as well as several other species (Rehbein and Oehlenschlager, 1982). Hence the TVB measurements values do not necessarily reflect the mode of spoilage but rather the quality of fresh products.

Measurement of TVBA depends upon steam distillation of volatile amines. The concentration of TVB-N in freshly caught fish is typically between 5 and 20mg N/100 g muscle, whereas levels of 30– 35mg N/100 g muscle are generally regarded as the limit of acceptability for ice stored temperate water fish (Huss 1988a; Connell, 1995).

3 MATERIALS AND METHODS

3.1 STUDY AREA AND SAMPLING PLAN

This work was done at NFAQCL-Nyegezi. The study was conducted in three phases. Phase I study was done on microbiology of on lake water, sediments and newly caught Nile perch, trials 1-3 respectively, from the fishing grounds. Phase II study consisted of two trials; trial 1 on the shelf life of laboratory iced whole Nile perch and trial 2 on chilled Nile perch fillets. While phase III study was composed of trial 1 on microbiology of whole Nile perch swabs and on line fillets, and trial 2, shelf life of establishments chilled Nile perch fillets.

Phases I and II samples were procured from Lake Victoria along Mwanza gulf by trawling method using Nyegezi Fresh Water Fisheries Training Institute (NFFI) MV Mdiria boat. Mwanza gulf is a reserved breeding area, figure 2. Trawling was covered between latitude $2^{\circ} 28' S$ and $2^{\circ} 32' S$; and longitude $32^{\circ} 49'E$ and $32^{\circ} 50'E$ at an average depth of 20 meters. Samples of whole Nile perch, sediments and water were all sampled at the same time during trawling. The phase III samples; whole Nile perch swabs, on line fillets and establishments chilled Nile perch fillets were procured from four establishments (E1; E2; E3 & E4) located in Mwanza City. All samples were brought and stored at the laboratory chill room/refrigerator set at 0 to $3^{\circ}C$ prior sub sampling laboratory test samples for subsequent analyses.

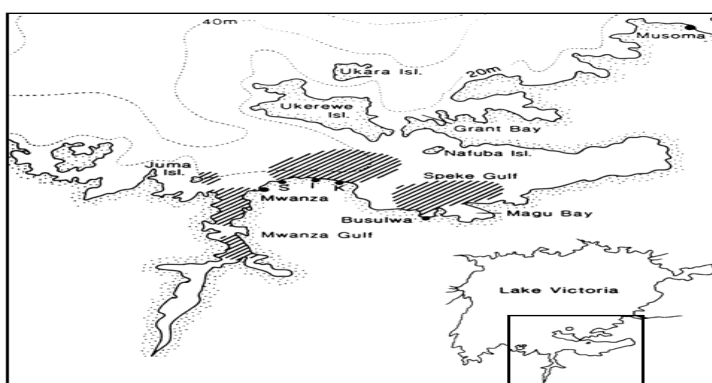
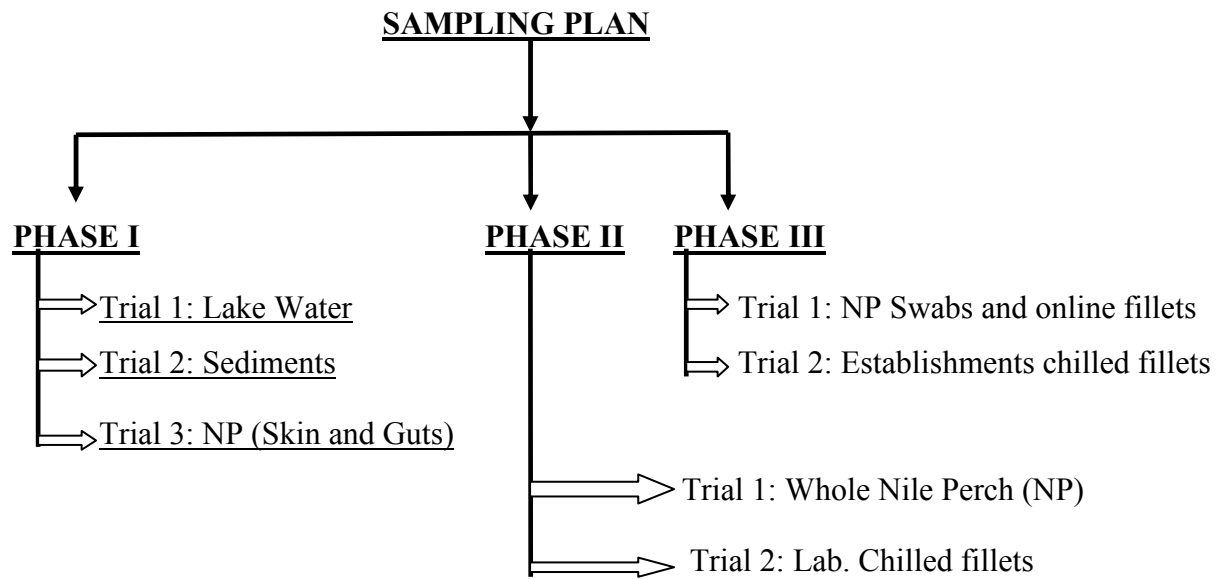


Figure 2: Sketch map of the South-East part of Lake Victoria. Fishing grounds of the various landing sites are indicated by shading. S: Semba; I: Igombe and K: Kayenze, (FAO, 2009).



Note: NP = Nile Perch

3.2 SAMPLING

3.2.1 Phase I: Microbiology of Water, Sediments and Nile Perch.

Samples of Water, Sediments and whole Nile perch were sampled along side with subsequent trawls in Mwanza gulf. Total of five trawls were done during sampling and two samples for each type were sampled from each trawl.

3.2.1.1 Phase I-trial 1: Water

Total of 5 duplicates (10) samples of Lake Water were sampled from five trawls using a sterile 250ml bottle. A bottle was tied up with a rope around the neck and directly released to fetch water from the surface. Immediately the bottle with water sample was placed with a cap and properly closed. The samples were stored in a cool box with ice and transported to the laboratory, then stored overnight in the laboratory refrigerator and analysed within 24 hours from sampling the following day.

3.2.1.2 Phase I- trial 2: Sediments

Sediments were scooped from the bottom of the lake using crab sampler and placed on sterile stainless steel tray at same trawls concurrent with water samples. Then by using sterile spoon a sample of sediments was put into sterile 250ml bottles. Immediately the bottle with sample was placed with a cap and properly closed. Two samples were sampled from each trawl to make total of 5 duplicates (10) samples. All samples were stored in a cool box with ice and transported to the laboratory, then stored overnight in the laboratory refrigerator and analysed within 24 hours from sampling the following day.

3.2.1.3 Phase I – Trial 3: Whole Nile perch

Similarly total of 5 duplicates (10) samples (fishes) of whole Nile perch each with an average weight of 2 kg were sampled, two fishes from each trawl, individually placed into a plastic bag and then stored in ice. Samples were transported to the laboratory, stored overnight in ice in the laboratory chill room prior to analysis the following day.

3.2.2 Phase II: Shelf life for Laboratory iced whole Nile perch and chilled fillets

3.2.2.1 Phase II trial 1: Shelf life of iced whole Nile perch

Samples (70 fishes) of whole Nile perch (*Lates niloticus*) (2-5 kg) were sampled from five trawls along Mwanza gulf fishing ground in Lake Victoria and iced immediately after each trawl (catch). Each trawl lasted for 30 minutes. Whole Nile perch samples were selected and iced on spot and then transported to the laboratory. At the laboratory samples were re-iced properly to a ratio of 1:1 (fish to ice) in 300ltr plastic tub with lid. The plastic tub was kept at the laboratory chill room maintained at 0-3°C during the shelf life study.

3.2.2.2 Phase II trial 2: Shelf life of Laboratory chilled fillets

Fifty (50) samples of whole Nile perch samples from the 5 trawls above were filleted at a fish processing establishment and the fillets were chilled in an air-blast freezer to 0±1°C before

being packed in Styrofoam boxes according to normal operating procedures at the establishment. Ten (10) Styrofoam boxes each with about 6-8 pieces of chilled fillets were taken to the laboratory and stored in a laboratory refrigerator at 0-3°C during the shelf life study.

3.2.3 Phase III: Microbiology of whole Nile perch swabs, on line fillets and shelf life of establishments chilled fillets

Samples were collected from four establishments (E1, E2, E3, & E4). The whole Nile perch (fishes) were iced and stored in fish transportation boats until the day of filleting at the establishments. The fishes were caught from canoes and brought to transportation boats, approximately 4-6 hours after catch. The number of storage days in ice from the day of fishing until filleting at establishments E1, E2, E3 and E4 were 10, 17, 6 and 5 respectively. All samples for each trial at each establishment collected corresponded to one batch/lot of raw material (whole Nile perch) in one transportation vessel.

3.2.3.1 Phase III – trial 1: Microbiology of whole Nile perch swabs and on line fillets

Four (4) swabs samples were taken from whole Nile perch, two before and two after washing from the processing line of the establishments. Sterile swab sticks were used for sampling from a prescribed area of 50cm² of skin surfaces using a template and then immediately placed in to a vial (bottle) with 10ml diluent (0.1% peptone and 0.85% salt solution) and stored in a cool box with ice bags until analysed at the laboratory. Also four (4) on line skinless fillet (SLF) samples were sampled, two before wash and two after wash at each establishment. Each of the four fillets sampled was put into individual polythene bag and stored in a cool box with ice bags. All samples were transported to the laboratory and stored in a laboratory refrigerator until analysed within 24 hours after sampling. However, no swabs and on line samples from establishment E4 were sampled because there was no production in progress. Therefore only the final packaged chilled fillets were sampled at establishment E4 (see Phase III – trial 2).

3.2.3.2 Phase III – trial 2: Shelf life of establishments chilled Nile perch fillets

Two samples (4-6 kg Styrofoam boxes) with newly processed fillets were sampled from a specific lot/batch at each establishment (E1, E2, E3, & E4) and transported to the laboratory and stored in the laboratory refrigerator at 0-3°C until analyzed.

3.3 PROCEDURES

In all samples mentioned above, analyses employed include sensory assessment on whole Nile perch (phase I trial 1), microbiological analysis on all samples above and chemical analysis on whole Nile perch and fillets. Sensory assessment, microbiological analysis and the preparation of chemical extracts for the TVB-N analysis were all carried out at NFQCL-Nyegezi, SANAS accredited testing facility and the TVB-N analysis were done at Sokoine University of Agriculture (SUA), Morogoro, Tanzania.

3.3.1 Sensory (organoleptic) assessment

Samples of iced whole Nile perch were sampled on different days: 4, 7, 10, 13, 15, 20, 22, 26, 28, 30 and 33 storage days in laboratory refrigerator at 0-3°C, see appendix III -A. Organoleptic assessment was carried out using a checklist (table 10) according to freshness ratings by applying a modified quality assessment scheme used to identify the quality index (QI) demerit score by Larsen *et al.*, (1992), see appendix 1. The scheme employed the main three quality parameters; namely general appearance, eyes and gills against the following characters (attributes): Skin, blood spot on gill cover, belly and smell; eye clarity; and gills colour and smell respectively. The excellent freshness quality fish ranked with quality index demerit score between 0-1, fish of good quality 1-2 and stale/spoiled fish had scores of 2-3. Three samples of iced whole Nile perch from phase II- trial 1 were sampled and placed for assessment each day of sampling. The panellists were comprised of untrained panellists from NFQCL-Nyegezi and NFFI staff. The number of panelists varied from three to maximum of seven.

Table 10: Freshness ratings using the quality assessment scheme used to identify the quality index demerit score (Larsen *et al.* 1992) modified.

Quality parameter	Character	Score (ice/seawater)			
		0	1	2	3
General appearance	Skin	Bright, shining	Bright, <i>cloudy mucus</i>	Dull, <i>Milky mucus</i>	<i>Dull, opaque mucus</i>
	Bloodspot on gill cover	None	Small, 10-30%	Big, 30-50%	Very big, 50-100%
	Stiffness	Stiff, in <i>rigor mortis</i>	Elastic	Firm	Soft
	Belly	Firm, normal <i>bulged</i>	<i>Firm/flat concave</i>	Soft	<i>Belly burst</i>
	Smell	Fresh, seaweed/metallic	Neutral	Musty/sour	Stale meat/rancid
Eyes	Clarity	Clear, <i>convex, bright pupil</i>	Cloudy, <i>dull pupil</i>	<i>Flat, opaque pupil</i>	<i>Concave, Grey pupil</i>
Gills	Colour	Characteristic, red <i>/bright colour, no mucus</i>	Faded, less discoloured, <i>Traces of mucus</i>	<i>Discoloured, Opaque mucus</i>	<i>Yellowish, Milky mucus</i>
	Smell	Fresh, seaweed/metallic, rancid	Neutral	Sweaty/slightly rancid	Sour stink /stale
Sum of scores	minimum 0 and maximum 24				

Legend: Italics words added (modified)

3.3.2 Microbiological analysis

3.3.2.1 Phase I: Natural microbiology of Water, Sediments and Nile perch.

3.3.2.1.1 Phase I-trial 1: Water

The following microbiological parameters were examined:

- Total Viable Counts (TVC) as per NMKL method #86, 4th ed., 2006
- *Enterobacteriaceae* as per NMKL method #144, 3rd ed. 2005
- *Escherichia coli* as per NMKL method # 125 4th ed. 2005
- *Salmonella* as per NMKL method # 71, 5th ed. 1999 and
- Pathogenic *Vibrio* species as per NMKL method # 156, 2nd ed. 1997

The undiluted water samples in bottles were thoroughly mixed by hand and made up to 1/100 dilution using sterile diluent.

- **Total viable counts (TVC):** Volumes of 1ml from undiluted, 1/10 and 1/100 dilutions were pour-plated in duplicates with plate count agar (PCA) (Oxoid CM0325B) tempered at 45⁰C and thoroughly mixed. The plates were left to solidify and incubated in an incubator set at 22⁰C for 72 hours. After incubation all colonies were counted and the results reported as cfu/ml.
- **Enterobacteriaceae:** Volumes of 1ml from undiluted, 1/10 and 1/100 dilutions were pour-plated in duplicates with 10ml of Violet Red Bile Glucose Agar (VRBGA) (Oxoid CM0485B) tempered at 45⁰C and thoroughly mixed. The plates were left to solidify and again added an overlay of 15ml VRBGA and allowed to solidify. The plates were then incubated in an incubator set at 30⁰C for 24 hours. After incubation all typical colonies were counted and if possible up to 10 typical colonies were purified for confirmation on Nutrient agar (Oxoid CM0003B) incubated at 37⁰C for 24 hours. After incubation purified colonies were tested for oxidase. *Enterobacteriaceae* are oxidase negative and typically oxidase negative colonies were reported as cfu/ml *Enterobacteriaceae*.
- **Escherichia coli:** Volumes of 1ml from undiluted, 1/10 and 1/100 dilutions were pour-plated in duplicates with 5ml of Tryptose Soya Agar (TSA), (Oxoid CM0131B) tempered at 45⁰C and thoroughly mixed. The plates were left to solidify and incubated at room temperature at 22-24⁰C for 2 hours, then added an overlay of 15ml Violet Red Bile Lactose Agar (VRBLA) (Oxoid CM0107B) and allowed to solidify. The plates were incubated in an incubator at 44⁰C ± 0.5⁰C for 24 hours. After incubation all typical (dark red) colonies were counted and if possible up to 10 typical colonies were confirmed in *Escherichia coli* (EC) broth (Oxoid CM0853B) at 44⁰C ± 0.5⁰C for 24 hours in a water bath. After incubation the gas positive tubes were inoculated by using sterile loop into tryptone broth tubes (Oxoid CM0087B) and incubated at 44⁰C ± 0.5⁰C for 24 hours. Following incubation, 0.5ml of Kovacs indole reagent was added into the tryptone broth tubes (TB). TB tubes which developed red ring were Kovacs indole positive and the calculated results were reported as cfu/ml presumptive *E. coli*.
- **Salmonella detection:** 25g (equivalent to 25ml) of undiluted water sample was weighed and added to 225ml of buffered peptone water (BPW) (Oxoid CM0509B) for pre-enrichment at 37⁰C for 24 hours. After incubation, 0.1ml of the enrichment was transferred to Rappaport-Vassiliadis (RV) medium (Sigma) and incubated at 42⁰C/24

hours. After incubation a loopfull of RV medium was streaked on to prepared plates of xylose lysine desoxycholate (XLD) agar (Oxoid CM0469B) and Bismuth sulphite agar (BSA), (Oxoid CM0201B) media. The plates were incubated at 37⁰C for 24 hours and for 48 hours for negative plates. Using a sterile loop, colonies from positive XLD/BSA plates were streaked for purification on nutrient agar (NA) plates and incubated at 37⁰C for 24 hours. After purification, using a sterile needle (straight loop), colonies were inoculated into Triple Sugar Iron (TSI) agar (Oxoid CM0277B) – butt and slant respectively. TSI tubes were incubated at 37⁰C for 24 hours. Positive TSI slants which produced gas with black butt and red slant were reported as presumptive *Salmonella* in 25ml of water samples.

- **Pathogenic *Vibrio* species detection:** 25g (equivalent to 25ml) of water was weighed and added to 225ml of alkaline peptone water (APW) (Oxoid CM1028B) for enrichment at 37⁰C for 24 hours. After incubation a loopfull of APW enrichment was streaked onto prepared plates of selective Cholera TCBS medium (Oxoid CM0333B). The TCBS plates were incubated at 37⁰C for 24 hours. After incubation suspicious colonies were flat-yellow for the *Vibrio cholerae* (3-5mm-diameter) or blue- green for either *Vibrio parahaemolyticus* (3-5mm -diameter) or *Vibrio vulnificus* (2-3mm-diameter). Suspected colonies were sub-cultured by streaking on TSA agar plates for purification and incubated at 37⁰C for 24 hours. After incubation, pure colonies were subjected to biochemical tests in Hugh and Leifson (oxidation/fermentation (O/F)) test and TSI agar slants. After incubation at 37⁰C for 24 hours presumptive results were read on O/F test tubes which produced a yellow colour with oxidation and or fermentation; and on TSI slants that developed a yellow colour without gas as presumptive *Vibrio cholera* in 25ml of water samples.

3.3.2.1.2 Phase I- trial 2: Sediments

Twenty grams (20g) of sediment samples was weighed into a stomacher bag and mixed with 180ml of diluent. The mixture was stomached by 400 stomacher for 30 seconds to make 1/10 dilution. Subsequent dilutions up to 1/1000 were made. The above (3.3.2.1.1) described test procedures used for analysis of water were all followed.

3.3.2.1.3 Phase I – Trial 3: Whole Nile perch

Volume of 200ml (equivalent to 200g) of diluents (0.1% peptone and 0.85% salt solution) was used to rinse individual fish samples placed in polyethylene bags. The rinse solutions were treated as undiluted test samples. Volumes of 25ml of rinse solutions were added to 225ml of Buffered peptone water (BPW) enrichment medium for detection of *Salmonella*. Similar volumes (25ml rinsed diluents) were added to 225ml of Alkaline peptone water (APW) enrichment medium for pathogenic *Vibrio* spp. Subsequent analytical steps (in 3.3.2.1.1.) as for water analyses above were then followed for the *Salmonella* and *Vibrio* analysis.

For examination of TVC and *Enterobacteriaceae* in fish guts 20g of fish guts were weighed into 180ml of diluents and stomached in 400 stomacher for 1 minute to make 1/10 dilution. Serial dilutions up to 1/1000 were made. Then subsequent analytical steps as for water and sediments analyses (3.3.2.1.1 and 3.3.2.1.2) used above were then followed.

3.3.2.2 Phase II: Laboratory- shelf life for iced whole Nile perch and chilled Nile perch fillets

3.3.2.2.1 Phase II trial 1: Shelf life of iced whole Nile perch

Samples were sampled on different days: 4, 7, 10, 13, 15, 20, 22, 26, 28, 30 and 33 storage days in laboratory refrigerator at 0-3°C, see appendix III -A. Whole Nile perch samples were sampled and put into polyethylene bag, then stored in the laboratory refrigerator at 0-3°C prior to analysis. The laboratory samples were prepared according to NMKL method #91, (2002). Plate Count Analysis was done according to the Iron agar (IA) method, (Gram *et al.*, 1987). The composition of the IA agar consists of the following ingredients:

- Peptone bacteriological (Oxoid LP0037B)
- Bacteriological agar –No.1 (Oxoid LP00113B)
- Yeast extract granules (Merck VM284953)
- Beef extract powder (Highmedia RM002)
- Ferric citrate salt
- Sodium thiosulphate
- Sodium chloride

Percentage (w/w) of IA constitutes used were the same as used by Gram *et al.*, (1987), see appendix II.

The *Enterobacteriaceae* analysis was carried out in accordance with the NMKL method #144 3rd ed. 2005 using VRBGA. The test samples were prepared by weighing out twenty grams (20g) of skin from both sides of whole Nile perch into a stomacher bag and mixed with 180ml of diluents. The mixture was homogenized by using the stomacher 400 for 1 minute to make 1/10 dilution. Further dilutions were made up to the highest dilution required during the shelf life study. Initially dilutions were 1/10 to 1/1000 and then increased up to the highest dilution of 1/10⁹ at the end of the storage time.

- **Total viable counts (TVC) and SSO:** Volumes of 1ml from serial dilutions were pour-plated in duplicates with Iron agar (IA) tempered at 45⁰C and thoroughly mixed. The plates were left to solidify and again added an overlay of approximate 15ml IA and allowed to solidify. The plates were then incubated in an incubator at 22⁰C for 72 hours. After incubation, both typical black and white colonies were counted and the results were reported in cfu/g. The black (SSO) colonies were calculated and reported separately as cfu/g. The sum of black and white colonies was reported as total viable counts in cfu/g.

Five (5) typical black colonies were purified on Nutrient agar (Oxoid CM0003B) and incubated at 37⁰C for 24 hours. After incubation, purified colonies were tested for oxidase, catalase, Gram reaction and glucose fermentation (O/F) in Hugh and Leifsson medium.

- **Enterobacteriaceae/SSO:** Volumes of 1ml from serial homogenate dilutions were pour-plated in duplicates with 10ml of VRBGA (Oxoid CM0485B) tempered at 45⁰C and thoroughly mixed. The plates were left to solidify and again added an overlay of 15ml VRBGA and allowed to solidify. The plates were then incubated in an incubator at 30⁰C for 24 hours. After incubation all typical (pink to red) colonies were counted and 10 colonies were purified on Nutrient agar (Oxoid CM0003B) incubated at 37⁰C for 24 hours. After incubation, purified colonies were tested for oxidase. *Enterobacteriaceae* are oxidase negative and typical oxidase negative colonies were reported as cfu/g *Enterobacteriaceae*. Further tests for catalase, Gram reaction and glucose fermentation (O/F) in Hugh and Leifsson medium were performed for ascertaining for SSO.

3.3.2.2.2 Phase II trial 2: Shelf life of chilled Nile perch fillets

Samples were sampled on different days: 2, 6, 10, 12, 15, 17, 19, 21 and 23 during storage in laboratory refrigerator at 0-3°C, see appendix III -C. The analyses for both TVC/SSO and *Enterobacteriaceae* for the chilled fillets used the same procedures as used in 3.3.2.2.1 above. However twenty gram (20g) of chilled skinless fillets from different areas was weighed into stomacher bag followed by 180ml of diluent and then stomached in the stomacher 400 for 1 minute to bring 1/10 dilution. Further dilutions were made up to the highest dilution required.

3.3.2.3 Phase III: Microbiology of whole Nile perch swabs, on line fillets and shelf life of establishments chilled Nile perch fillets

3.3.2.3.1 Phase III– trial 1: Microbiology of whole Nile perch swabs and on line fillets

Whole fish swabs: Swabs were analysed for TVC using Iron agar and *Enterobacteriaceae* using VRBGA. Swabs were thoroughly mixed by hand with the 10ml diluents in the swab vial and serial dilutions up to 1/1000 were made. The same procedures were then used as described in 3.3.2.2.1 for Total viable counts (TVC) and SSO using Iron agar and for *Enterobacteriaceae* using VRBGA. The counts were calculated from the actual counts per ml (cfu/ml) multiplied by 10mls of used diluents and divided by 50cm² swabbed area. Then the counts were reported in cfu/ cm² or logcfu/cm².

On-line fillets: The same procedures were used as in part 3.3.2.2 above.

3.3.2.3.2 Phase III – trial 2: Shelf life of establishments chilled Nile perch fillets

Samples for establishments E1 and E2 were sampled on different days: 2, 8, 12, and 15 during storage in laboratory refrigerator at 0-3°C, see appendix III –D. Samples from establishments E3 and E4 were sampled from days: 2, 6, 10, 13, 15 and 17 during storage in laboratory refrigerator at 0-3°C, see appendix III –E. The same procedures above for TVC/SSO and *Enterobacteriaceae* as in part 3.3.2.2.2 were used.

3.3.3 Chemical analysis

3.3.3.1 Total volatile base nitrogen (TVB-N) and pH

TVB-N was measured by using steam distillation method according to Malle, P. and Poumeyrol, M. (1989). Whole Nile perch skin was removed and 100g of muscle/fillets were weighed into a laboratory blender followed by 200ml (equivalent to 200g) of 7.5% Trichloroacetic Acid (TCA) and blended. About 75-100ml filtrate was filtered by using Watman filter paper number 3. The extracts/filtrates were stored in laboratory refrigerator at 0°C for 1-2 months prior analysis. Volume of 25ml of filtrate was put into a distillation flask and 6ml 10% alkaline (NaOH) added. The mixture was distilled and generated free bases (distillates) which were collected into 250ml conical flask containing 10ml of 4% Boric acid (with 0.04ml mixture of methyl red and bromocresol green indicator). The volatile bases were titrated against 0.025N Sulphuric acid and the amount of Sulphuric acid (H₂SO₄) used was measured as total bases distilled over. The bases (distillates) include mixture of ammonia, trimethylamine and dimethylamine each contain one basic nitrogen atom per molecule. The TVB-N was calculated and expressed as mg nitrogen/100g of fish muscle using equation: $TVB-N_{mg/100g} = \frac{[14mg/mol \times A \times B \times 300]}{25ml}$. Whereas A = ml of sulphuric acid and B = normality of sulphuric acid.

The pH was measured from the homogenate of fish muscle suspended in distilled water using thermo Orion pH meter.

3.3.4 Data analysis

Data and results were statistically computed using normal Microsoft office excel 2007. The colony forming units (cfu) per gram/ml/cm² were all converted to log₁₀ colony forming units (logcfu) per g/ml/cm² of sample for subsequent data analysis. Data in logcfu per g/ml/cm² forms were presented by tabular and graphs forms.

4 RESULTS

4.1 PHASE I: MICROBIOLOGY OF WATER, SEDIMENTS AND WHOLE NILE PERCH

4.1.1 Phase I trial 1: Water

All results from samples of surface lake water were very low in total viable counts and *Enterobacteriaceae* or zero in numbers (cfu) per ml (table 11). Only one sample among the five samples was presumptive *E. coli* in Kovacs (indole) reagent which developed a red ring.

Table 11: Microorganisms of surface lake water

PARAMETERS	SAMPLES (#TRAWLS)					RANGE	
	1	2	3	4	5		
Total viable count	cfu/ml	20	0	13	0	19	0-20
<i>E. Coli</i>	cfu/ml	1	0	0	0	0	0-1
<i>Enterobacteriaceae</i>	cfu/ml	3	2	0	5	3	0-5

One sample was presumptive *V. cholerae* per 25ml of water in TSI (table 12) and *V. parahaemolyticus/V. vulnificus* were absent in 25ml of water. Also *Salmonella* was absent in 25ml of water.

Table 12: Pathogenic microorganisms of surface water

PARAMETERS	SAMPLE SIZE	NUMBER OF SAMPLES	PRESUMPTIVE SAMPLES
<i>Vibrio cholera</i>	25ml	5	1 (20%)
<i>Vibrio parahaemolyticus/</i> <i>Vibrio vulnificus</i>	25ml	5	0 (0%)
<i>Salmonella</i>	25ml	5	0 (0%)

4.1.2 Phase I trial 2: Sediments

The microbiological counts for sediments were between 2.5×10^3 - 2.2×10^4 cfu/g of sediment (table 13). These counts may imply that sediments from the bottom of water have likelihood of being good reservoir for many microorganisms. There was no *Enterobacteriaceae* and *E. coli* detected in the samples. Thus, it may be seemingly, the *Enterobacteriaceae* and *E. coli* can not survive for long time in aquatic environment (sediments) as compared to microorganisms represented in the TVC.

Table 13: Microorganisms of sediments

PARAMETERS	SAMPLES (#TRAWLS)						
	1	2	3	4	5	RANGE	
Total viable count	cfu/g	3500	22000	6100	2500	2800	2.5×10^3 - 2.2×10^4
<i>E. Coli</i>	cfu/g	0	0	0	0	0	0
<i>Enterobacteriaceae</i>	cfu/g	0	0	20	10	0	0-20

The pathogenic *Vibrios ssp.* (*V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*) as well as *Salmonella* were absent in 25g of sediments (table 14).

Table 14: Pathogenic microorganisms of sediments

PARAMETERS	SAMPLE SIZE	NUMBER OF SAMPLES	PRESUMPTIVE SAMPLES
<i>Vibrio cholera</i>	25g	5	0 (0%)
<i>Vibrio parahaemolyticus/</i>			
<i>Vibrio vulnificus</i>	25g	5	0 (0%)
<i>Salmonella</i>	25g	5	0 (0%)

4.1.3 Phase I trial 3: Whole Nile perch

All pathogenic *V. cholerae*, *V. parahaemolyticus/ V. vulnificus* were presumptively absent in 25ml of wash diluent of whole Nile perch (table 15). However, there was presumptive *Salmonella* in 25ml of wash diluent from one sample of Nile perch skin.

Table 15: Pathogenic microorganisms on Nile perch skin.

PARAMETERS	SAMPLE SIZE	NUMBER OF SAMPLES	PRESUMPTIVE SAMPLES
<i>Vibrio cholerae</i>	25ml	5	0 (0%)
<i>Vibrio parahaemolyticus</i> / <i>Vibrio vulnificus</i>	25ml	5	0 (0%)
<i>Salmonella</i>	25ml	5	1 (20%)

The results from the total viable counts (TVC) of the Nile perch gut samples ranged from 2.4×10^2 - 1.0×10^6 cfu/g (table 16). It can be concluded that the fish guts are good reservoirs of indigenous micro flora that may contribute to contamination during processing if handlers do not abide to good hygiene and operating practices. The results for *Enterobacteriaceae* ranged from 4.0×10^3 - 3.8×10^4 cfu/g. The results indicate that the fish guts can be an important reservoir of bacteria contamination of Nile perch during processing at establishments.

Table 16: Microorganisms of Nile perch guts

PARAMETERS		SAMPLES (TRAWLS)					RANGE
		1	2	3	4	5	
Total plate count	cfu/g	16000	1000000	240	580	240000	2.4×10^2 - 1.0×10^6
<i>Enterobacteriaceae</i>	cfu/g	17000	38000	0	6000	4000	4.0×10^3 - 3.8×10^4

One sample was presumptive *V. cholerae* per 25g of fish guts (table 17). The *V. parahaemolyticus* and or *V. vulnificus* were absent in 25g from all samples.

Table 17: Pathogenic microorganisms of Nile perch guts

PARAMETERS	SAMPLE SIZE	NUMBER OF SAMPLES	PRESUMPTIVE SAMPLES
<i>Vibrio cholera</i>	25g	5	1 (20%)
<i>Vibrio parahaemolyticus</i> <i>/Vibrio vulnificus</i>	25g	5	0 (0%)

4.2 PHASE II: SHELF LIFE OF LABORATORY ICED WHOLE NILE PERCH AND CHILLED FILLETS

4.2.1 Phase II trial 1: Shelf life of iced whole Nile perch

4.2.1.1 Sensory (organoleptic) assessment

Freshness quality for whole Nile perch was assessed by untrained panellists according to the freshness ratings using the quality assessment scheme used to identify the quality index (QI) demerit score by Larsen *et al.*, (1992). Figure 3: #1-3 shows belly, clear red coloured eyes and gills as well as characteristic bright and shining skin of whole Nile perch following 10 days storage in ice. Figure 3: # 4 shows spoiled whole Nile perch with general appearance of dull and opaque skin following 33 days at the end of storage time in ice. The main QIM score contributors (figure 5) were change of the general appearance on stiffness: from stiff (very fresh fish) then elastic, firm and soft (stale/spoiled fish) with average scored up to 2.50. Then followed by a general appearance of fish/gill smell: which were fresh/ seaweed/ metallic/ rancid for very fresh fish and stale meat/sour stick (stale/spoiled fish) both with average scores of ≥ 2 . The appearance of skin was changed from bright/shining (very fresh fish) to dull/opaque for stale/spoiled fish with average score of 1.67.

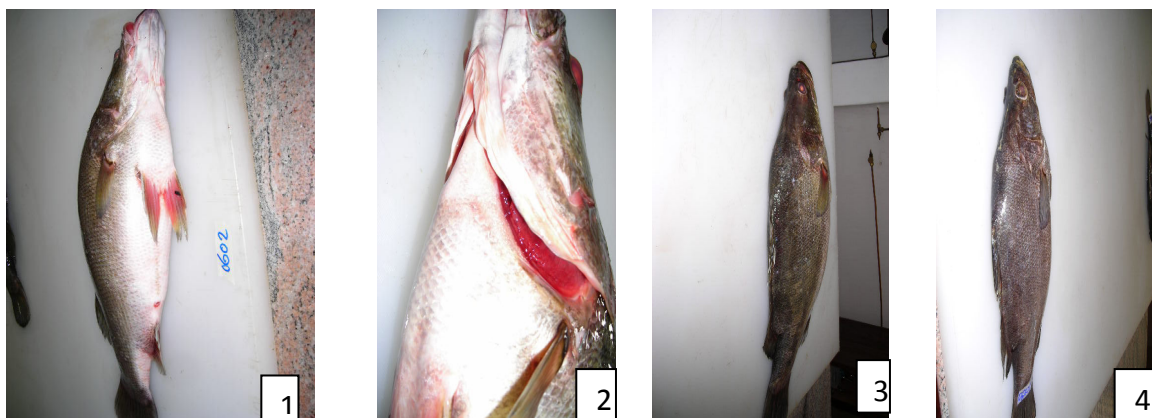


Figure 3: 10 day's whole Nile perch (#1-3) from left and 33 days whole Nile perch (#4-spoiled) right, respectively during storage in ice at laboratory chill room (0-3⁰C).

The excellent freshness fish grade (QIM score) was scored during 15 days and good quality fish were scored during 3-4 weeks of storage in ice. QIM scores characterized with a constant increase (from 2-14 score) through out the 33 days storage time (figure 4). The quality of fish after 3 weeks of storage in ice was observed and was graded as not fit for processing prime quality fresh chilled fillets for export.

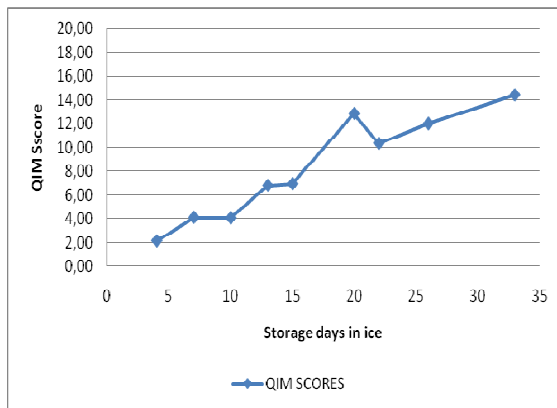


Figure 4: QIM score for whole Nile perch during storage in ice.

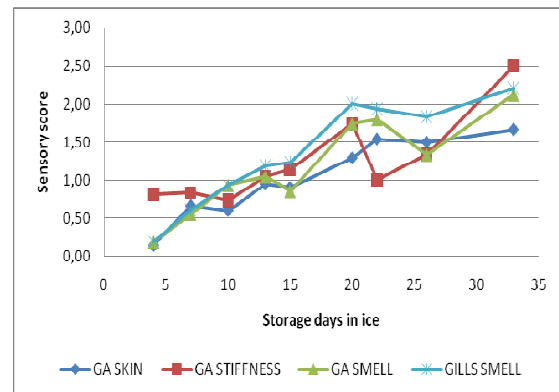


Figure 5: Main contributing attributes to QIM score for whole Nile perch during storage in ice, (GA: General appearance).

4.2.1.2 Microbiological analysis

The test results for specific spoilage organisms (SSO), total viable counts (TVC) and *Enterobacteriaceae* counts for Laboratory iced whole Nile perch stored in ice at 0-3°C are shown in figure 6. The SSO counts were low up to 4th day of storage in ice and then rapidly increased from 2.75 - 6.15 log₁₀ cfu/g after 15 days of storage. Similarly the TVC counts were low but higher than the SSO counts on the 4th day of storage but increased from 4.12 - 7.88 log₁₀ cfu/g after 15 days of storage. The *Enterobacteriaceae* counts were similar to TVC counts after 4 days of storage i.e. 4.40log₁₀ cfu/g and increased up to 6.22 log₁₀ cfu/g after 15 days of storage. These counts corresponded well to the excellent freshness quality scores for the same period of whole Nile perch for processing into very good quality fillets and or other products. After 15 days of storage and up to 22 days of storage of whole Nile perch in ice all microbiological counts slowly increased to 9.49log₁₀cfu/g (SSO); 9.84log₁₀cfu/g (TVC) and 8.22log₁₀cfu/g (*Enterobacteriaceae*). Then the counts increased up to a stationary phase during the fourth and fifth weeks of storage and reached maximum

counts of $>10\log_{10}\text{cfu/g}$ (SSO), $>10\log_{10}\text{cfu/g}$ (TVC) and $>9\log_{10}\text{cfu/g}$ (*Enterobacteriaceae*) on the skin.

The SSO counts were lower than the TVC counts at the beginning period (4 days) but after that the difference between these counts varied only by very small margins. Even with the high skin counts the fish still had a freshness good quality for processing after 22 days of storage for other products preferably frozen fillets. Whereas during the fourth week of storage in ice, the freshness of the fish was also evaluated fit for other Nile perch products markets or final consumers but not for processing prime fresh chilled Nile perch products for export.

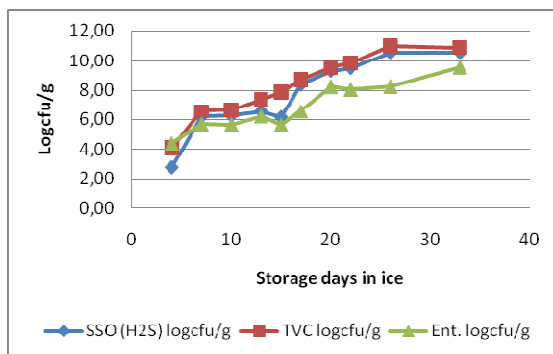


Figure 6: Changes of specific spoilage organism (SSO), total viable counts (TVC) and Enterobacteriaceae (Ent) in whole Nile perch during storage in ice.

The overall results on QIM score, spoilage micro flora and TVB-N during shelf life study in whole Nile perch stored in ice at $0-3^{\circ}\text{C}$ are shown in figures 7 and 8. Parallel trends were observed between all the results on the spoilage micro flora and QIM score implying that they are good quality parameters for decision making on rating freshness quality attributes for whole Nile perch stored in ice (figure 7). However, *Enterobacteriaceae* counts and TVB-N were parallel and both characterized by a constant increase during all four weeks (26-28 days) storage time (figure 8).

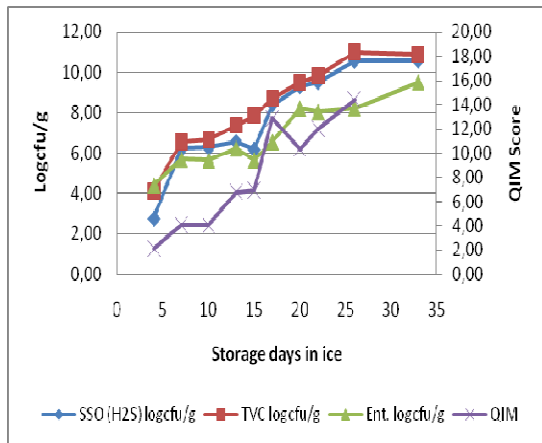


Figure 7: Changes of specific spoilage organism (SSO), total viable counts (TVC), *Enterobacteriaceae* (Ent.) and QIM score in whole Nile perch during storage in ice.

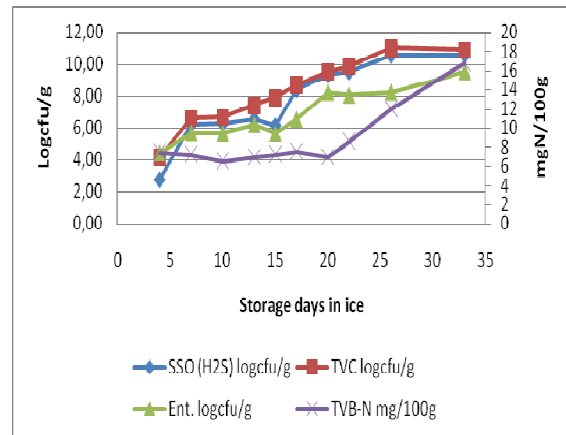


Figure 8: Changes of specific spoilage organism (SSO), total viable counts (TVC), *Enterobacteriaceae* (Ent.) and TVB-N in whole Nile perch during storage in ice.

4.2.1.3 Chemical analysis

The TVB-N results for whole Nile perch were stable with levels between 6-8 mgN/100g during the first three weeks of storage in ice (figure 9). Then the levels increased steadily up to 16.80mgN/100g after 33 days of storage. The relation between the TVB-N and QIM score changes during the storage time is shown in figure 10. Generally there is no parallel relation between these parameters implying that they can not be used as a complimentary pair to make decision for the freshness quality of fresh whole Nile perch stored in ice. QIM scores showed a continuous increasing trend as opposed to TVB-N for the first 3 weeks of storage. TVB-N showed a constant lag phase for 3 weeks and increasing phase from 20-33 days of storage. This implies that QIM score is better method than TVB-N values for evaluating the freshness quality of Nile perch during storage in ice. The pH of whole Nile perch skin shifted from 7.01 to 7.11 during the storage time of 33 days.

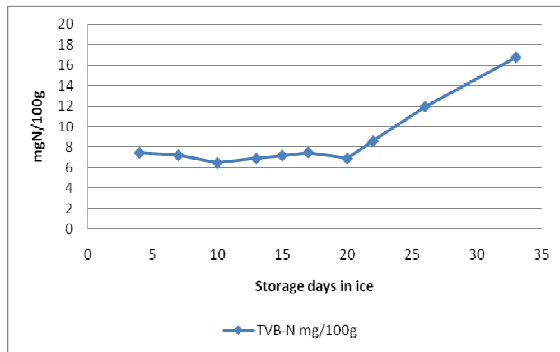


Figure 9: Changes of TVB-N in whole Nile perch during storage in ice.

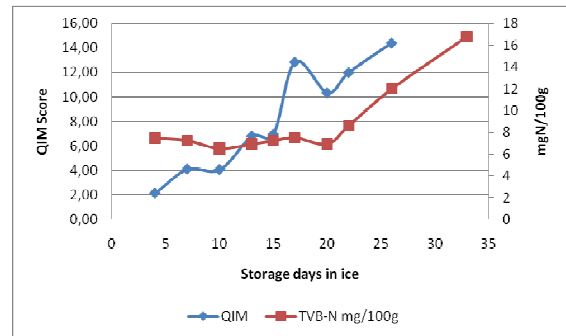


Figure 10: Changes of QIM and TVBN in whole Nile perch during storage in ice.

4.2.2 Phase II trial 2: Shelf life of laboratory chilled Nile perch fillets

4.2.2.1 Microbiological analysis

The maximum storage time of ice stored newly caught Nile perch fillets was 17-23 days. The freshness quality of the fillets was judged very good up to 17 storage days. The good quality fillets were accepted up 19-21 days and spoiled on the last two (22-23) storage days. The microbiological changes SSO, TVC and *Enterobacteriaceae* counts in chilled Nile perch fillets stored at laboratory refrigerator 0-3°C, processed from newly caught whole Nile perch after 2 days storage in ice. The SSO counts of fillets after 2 days were about 2 log₁₀ cfu/g and rapidly increased up to >5 log₁₀ cfu/g after 6 days of storage. Then decreased from 5.72-4.93 log₁₀ cfu/g up to 10 storage days, and increased again up to 8.35 log₁₀ cfu/g after 17 days of storage. TVC counts slowly increased from 4.42-6.48 log₁₀ cfu/g after 6 storage days and up to 8.54log₁₀cfu/g after 17 days of storage. The trend was different for *Enterobactereaceae* which showed a decrease in counts from 4.60-3.31 log₁₀ cfu/g after 6 days of storage and then slowly increased to 4.50 log₁₀ cfu/g after 17 days of storage. Finally all parameters increased up to a stationary phase during 17-23 storage days, 8.74-9.31log₁₀cfu/g (SSO), 9.61-9.67log₁₀cfu/g (TVC) and 8.10log₁₀cfu/g (*Enterobacteriaceae*) are shown in figure 11.

4.2.2.2 Chemical analysis

Changes of total volatile base nitrogen (TVB-N) in chilled Nile perch fillets stored at 0-3°C is shown in figure 12. The TVB-N showed a stable trend with low increase in the first 12 days of storage (6.72-7.67mgN/100g of fillet). Then the levels rapidly increased up to 26.92mgN/100g after 23 days of storage. The values are below the acceptability limits of 30-35mgN/100g. In view of these slow changes during the first 2 weeks and steady increasing after 23 days of storage time, the TVB-N is not a good indicator for making decision on freshness quality of chilled fillets. The change in pH was from 6.4 to 7.0 units during 23 days storage time.

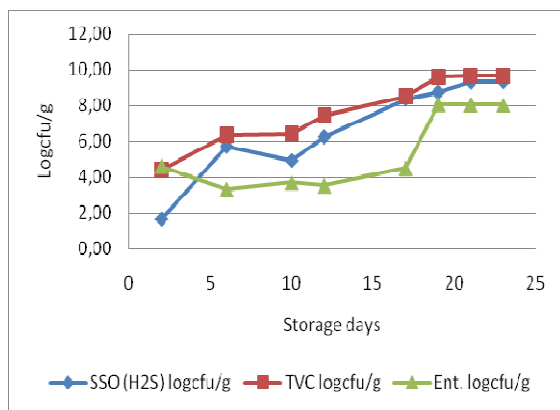


Figure 11: Changes of specific spoilage organism (SSO), total viable counts (TVC) and *Enterobacteriaceae* (Ent.) in chilled fillets from newly caught Nile perch.

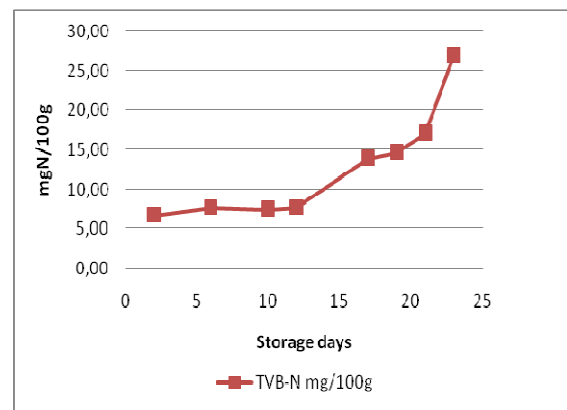


Figure 12: Changes of TVB-N in chilled fillets from newly caught Nile perch.

The specific micro flora (SSO, TVC) sharply increased at the beginning of storage and followed with a constant increase. The *Enterobacteriaceae* changes showed general lag phase at the first 2 weeks of storage but steady increase for the remaining storage period and chemical changes were stagnant during 13 days of storage (figure 13). Rapid changes occurred during 2-3 week of storage, then a constant /stationary phase were noted in all microbiological counts and TVB-N depicted exponential increase up to the end of storage time (23 days).

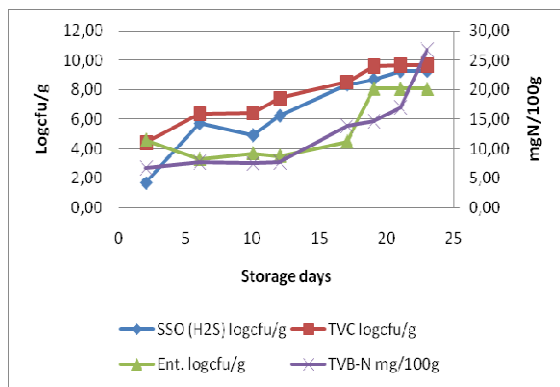


Figure 13: Changes of specific spoilage organisms (SSO), total viable counts (TVC), *Enterobacteriaceae* (Ent.) and TVB-N in chilled fillets from newly caught Nile perch.

4.3 PHASE III: MICROBIOLOGY OF WHOLE NILE PERCH SWABS, ON LINE FILLETS AND SHELF LIFE OF ESTABLISHMENTS CHILLED FILLETS

4.3.1 Phase III - Trial 1: Microbiology of whole Nile perch swabs and online fillets

Whole Nile perch is normally collected from various landing sites on shore and islands of Lake Victoria. Whole Nile perch is not washed nor gutted at fishing grounds/upstream. It is collected and iced in the transportation vessels, insulated boats and trucks at islands or landing sites. Usually collection vessels collect fish at minimum of 3 days to maximum of 3 weeks (21days), during this period the fish is stored in ice until transported to the establishments. The fish is off loaded and washed at the receiving section of the establishments. There are at least two washing stages. At the first stage the fish is scrubbed using brush (coloured) and washed with running/shower water. Then it is rinsed in next (second) stage by another handler/person and finally size graded and placed in a container (figure 14) with melting ice and stored chilled until they are subjected to alternative processes. The tables 18 and 19 below show the microbiological results for TVC and *Enterobacteriaceae* before (BW) and after washing (AW) of whole Nile perch and online fillets prior to the super chilling stage at three fish processing establishments (E1, E2, & E3).



Figure 14: Whole Nile perch in stainless container after washing at establishments.

The results of whole Nile perch swabs samples taken at receiving points of the establishments are shown in table 18. There was a significant reduction of microbiological loads in particularly for *Enterobacteriaceae* in all three establishments. The reduction for *Enterobacteriaceae* was between 1.04-2.20 log cfu/cm² in the three establishments. The reduction in TVC was greatest in E1 (1.48 log cfu/cm²) followed by E2 (0.5 log cfu/cm²) but increased slightly in E3 (-0.27 log cfu/cm²). The microbial loads in the three establishments BW for *Enterobacteriaceae* are 2.33–3.57 log cfu/cm² and for TVC from 5.08-6.12 log cfu/cm².

Table 18: Whole Nile perch swabs before and after wash at establishments.

SWABS BEFORE WASH (BW) AND AFFTER WASH (AW)						
ESTABL.	TVC – LOG CFU/cm ²			ENTEROBACTERIACEAE - LOG CFU/cm ²		
	BW	AW	BW-AW	BW	AW	BW-AW
E1	6,12	4,64	1,48	3,57	1,37	2,20
E2	5,48	4,98	0,50	2,33	1,29	1,04
E3	5,08	5,35	-0,27	3,49	1,76	1,73

The results of online skin less fillets taken from three establishments are shown in table 19. The *Enterobacteriaceae* counts BW ranged from 2.91-4.55 log₁₀cfu/g and 1.63-3.33 log₁₀ cfu/g AW. There was significant reduction for E2, 4.55-1.63 log₁₀ cfu/g BW and AW respectively, but no change for the E1 & E3. The results for TVC in all three establishments

BW were 5.49-6.27 log₁₀ cfu/g, less than 1 log₁₀ cfu/g different between the establishments. The TVC after washing was between 3.54-6.03 log₁₀ cfu/g, implying that there was a significant reduction in microbial loads between BW and AW. The (BW-AW) value for *Enterobacteriaceae* was between -0.02–2.93log₁₀cfu/g and TVC between 0.24-1.95log₁₀cfu/g in all three establishments.

Table 19: On line Nile perch fillets before and after washing at the establishments.

ONLINE FILLETS BEFORE WASH (BW) AND AFFTER WASH (AW)						
ESTABL.	TVC - LOGCFU/G			ENTEROBACTERIACEAE - LOGCFU/G		
	BW	AW	BW-AW	BW	AW	BW-AW
E1	6,27	6,03	0,24	3,31	3,33	-0,02
E2	5,49	3,54	1,95	4,55	1,63	2,93
E3	5,55	4,83	0,72	2,91	2,92	0,00

4.3.2 Phase III – Trial 2: Shelf life of establishments chilled fillets

4.3.2.1 Establishment E1 – chilled fillets (10 days iced whole Nile perch)

Nile perch chilled fillets were sampled from a lot of raw fish stored in ice for 10 days before being processed. Figures 16-18 shows the changes in spoilage micro flora and TVB-N in chilled fillets during shelf life study. SSO and TVC increase were considerably more than *Enterobacteriaceae* and TVB-N characterised no significant increase during the first 12 days. The changes in SSO and TVC in chilled fillets during the first 12 storage days after processing showed slow increase followed by a sharp increase between days 12-15. The *Enterobacteriaceae* remained more or less constant for the first 12 days of storage but increased sharply like SSO and TVC between days 12-15 (figure 16). The results depict that the SSO and TVC counts are generally high compared to the laboratory chilled fillets. The general observation here is that SSO and TVC counts are differing in very small margins implying that the SSO were already in high numbers following 10 days storage of whole Nile perch in ice. The level of *Enterobacteriaceae* counts are lower compared to SSO and TVC counts which is similar as observed with laboratory chilled fillets. Figure 17 shows chemical (TVB-N) changes during storage of chilled fillets. The results show very low increase from 6.55-8.65mgN/100g for up to 12 days of storage. Then the levels increased rapidly to

21mgN/100g from days 12-15 which correlates with similar increase in the microbiological counts for the same period. The changes in pH shifted from 6.7 to 7.0 units during all 15 days of storage.

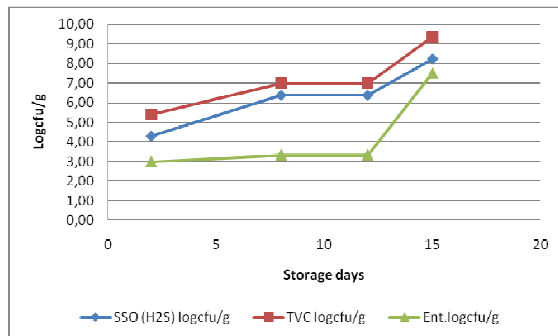


Figure 15: Changes of specific spoilage organisms (SSO), total viable counts (TVC) and *Enterobacteriaceae* (Ent.) on chilled fillets in E1.

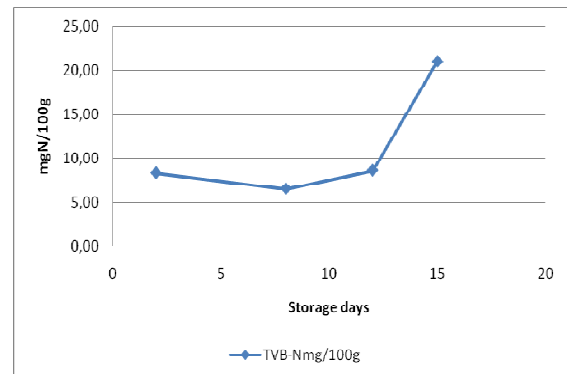


Figure 16: Changes of TVB-N on chilled fillets in E1

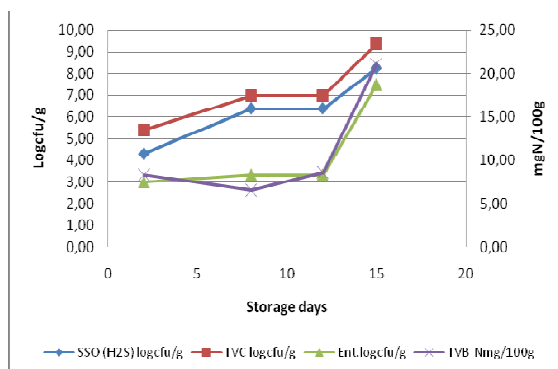


Figure 17: Changes of specific spoilage organism (SSO), total viable counts (TVC), *Enterobacteriaceae* (Ent.) and TVB-N on chilled fillets.

4.3.2.2 Establishment E2 – chilled fillets (17 days iced whole Nile perch)

Nile perch chilled fillets were processed and sampled from a lot of raw fish stored in ice for 17 days before processing. Establishment E2 showed a constant increase for the SSO and TVC counts during the first 12 days of storage after processing then followed by a rapid increase up to 15 days of storage (figure 18). The initial counts were $>4 \log_{10}$ cfu/g after 2 days of storage and increased in 1 week to about $7 \log_{10}$ cfu/g and then sharply increased to $\geq 10 \log_{10}$ cfu/g counts which were more than counts reached by spoiled Nile perch stored in ice for 33 days. The *Enterobacteriaceae* counts increased from 2-4 \log_{10} cfu/g up to the

end of the storage time. These results depict that from the beginning of storage after processing; the spoilage micro flora counts were generally high compared to the counts of laboratory chilled fillets. The interesting observation is that SSO and TVC were almost parallel implying that the SSOs were already in big numbers following the 17 days storage of whole Nile perch in ice. Figure 19 shows chemical (TVB-N) changes during storage of the chilled Nile perch fillets. The trend shows constant lag phase compared to the exponential changes of spoilage micro flora during the first 12 days of storage. The changes were very narrow between 5-7mgN/100g up to 12 days of storage, but increased to 10mgN/100g during 12-15 days of storage. The general trends shows rapid increase of SSO and TVC during the storage period, but moderate increase in TVB-N for the first 12 days and then exponentially increase from 12-15 days of storage. The changes in pH shifted from 6.62-6.82 units during the 15 days of storage.

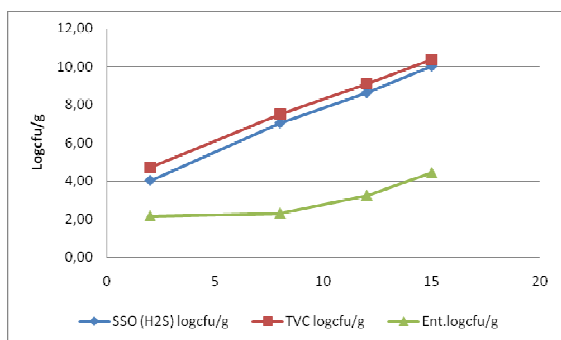


Figure 18: Changes of specific spoilage organism (SSO), total viable counts (TVC) and *Enterobacteriaceae* (Ent.) on chilled fillets.

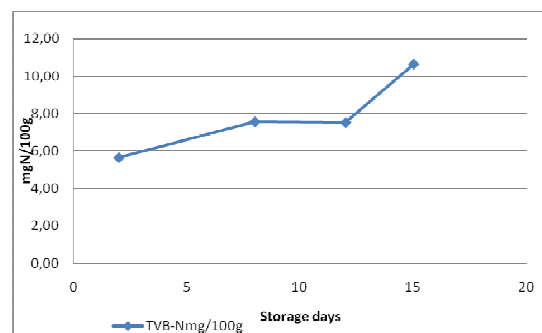


Figure 19: Changes of TVB-N on chilled fillets

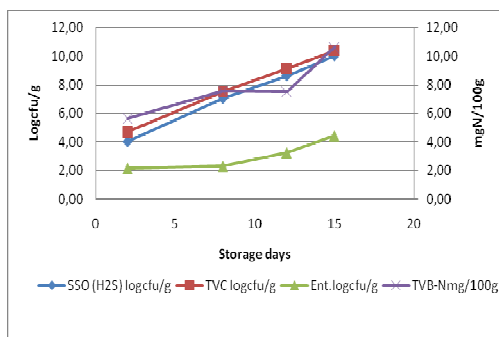


Figure 20: Changes of specific spoilage organism (SSO), total viable counts (TVC) and *Enterobacteriaceae* (Ent.) and TVB-N on chilled fillets.

4.3.2.3 Establishment E3 – chilled fillets (6 days iced whole Nile perch)

Chilled fillets were processed and sampled from a lot of whole Nile perch stored in ice for 6 days. Changes of spoilage micro flora on chilled fillets during shelf life study showed constant increase up to 15 days of storage (figure 23). The SSO and TVC counts produced parallel increase during the storage period but the TVB-N levels remained more or less unchanged for the first 13 days and then increased rapidly from days 13-17 of storage. The SSO and TVC increased from 3-8 log₁₀ cfu/g and the *Enterobacteriaceae* only from 2-3 log₁₀ cfu/g during 13 days of storage as can be seen in figure 21. After 17 storage days, the counts increased to >8 log₁₀ cfu/g (SSO), and >9 log₁₀ cfu/g (TVC) while there were no increase for *Enterobacteriaceae* counts. The TVB-N values remained constant between 5-7mgN/100g up to 15 storage days. It then increased to >10mgN/100g during days 15-17 of storage (figure 22). The TVB-N values were below the maximum acceptable limits. The changes in pH shifted within 6.8-7.3 units during all 17 storage days.

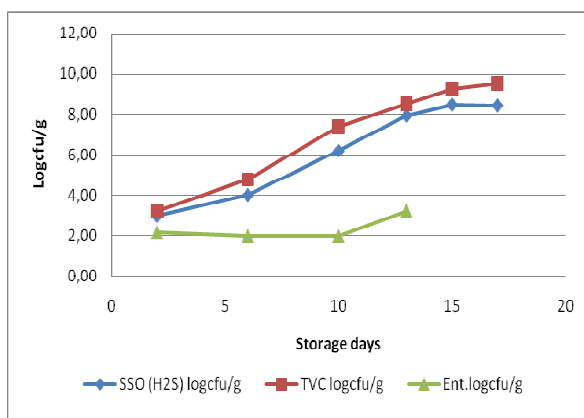


Figure 21: Changes of specific spoilage organism (SSO), total viable counts (TVC) and *Enterobacteriaceae* (Ent.) on chilled fillets.

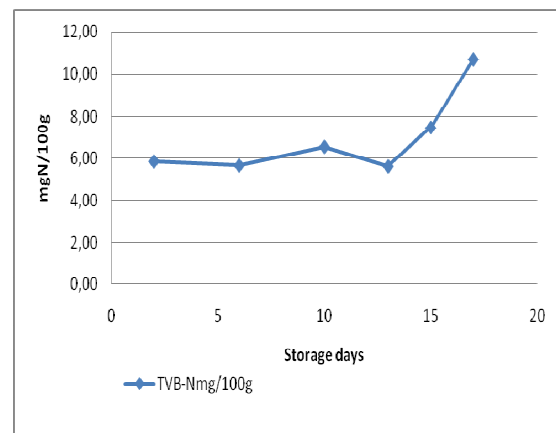


Figure 22: Changes of TVB-N on chilled fillets.

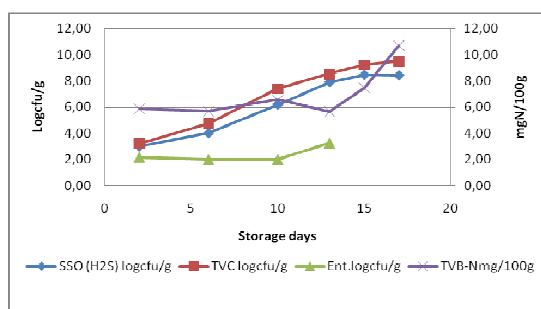


Figure 23: Changes of specific spoilage organisms (SSO) total viable counts, *Enterobacteriaceae* (Ent.) and TVB on chilled fillets.

4.3.2.4 Establishment E4 – chilled fillets (5 days iced whole Nile perch)

Chilled Nile perch fillets were processed from a lot of whole Nile perch stored in ice for 5 days. Changes for spoilage bacteria on chilled fillets were constant up to 13 storage days (figure 24 and 25). The trend was characterised by an increase for both SSO and TVC in parallel and reached a stationary phase after 13-15 storage days. The changes in TVB-N showed moderate increase from the beginning up to 13 days and then very rapid increase between days 13-15. The SSO and TVC increased from 3-7 log₁₀ cfu/g in the first 10 days of storage and to 9 log₁₀ cfu/g after 15 days while the *Enterobacteriaceae* counts gave only a slight increase from 2.5-3.7 log₁₀ cfu/g. Again these results indicate that the SSO counts are closely equals to TVC counts between 13-15 storage days. The chemical (TVB-N) changes during 15 days storage of chilled Nile perch fillets show an increase from about 5-13 mgN/100g up to 15 storage days (figure 25 and 26). The changes in pH shifted from 6.5-6.9 units during 15 storage days.

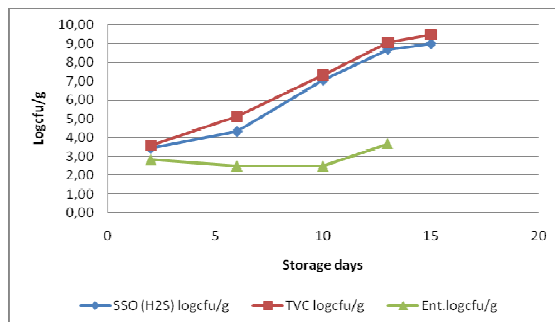


Figure 24: Changes of specific spoilage organism (SSO), total viable counts (TVC) and *Enterobacteriaceae* (Ent.) on chilled fillets.

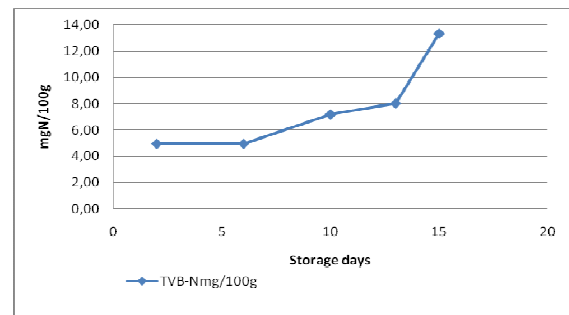


Figure 25: Changes of TVB-N on chilled fillets.

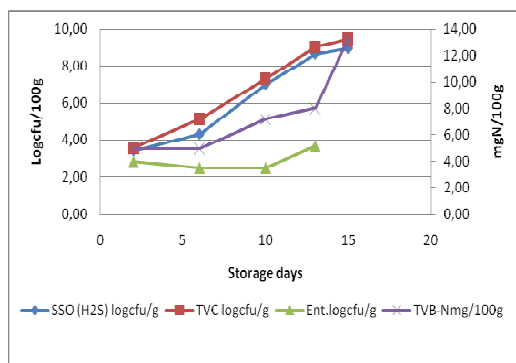


Figure 26: Changes of specific spoilage organism (SSO), total viable counts (TVC), *Enterobacteriaceae* (Ent.) and TVB-N on chilled fillets.

The results from chilled fillets (figure 27) from all four establishments show similar trends on both spoilage microflora and TVB-N. Changes in the spoilage micro flora on all chilled fillets during storage, initially were 3-4log₁₀cfu/g and at the end of storage time were ≥8-9log₁₀cfu/g (5 and 6 days in ice) and ≥10log₁₀cfu/g (10 and 17 days in ice) respectively. The changes in total volatile base nitrogen (TVB-N) were from 10-21mgN/100g, the values were well below the maximum limits of 30-35mgN/100g of acceptability of fresh fish. The trends in SSO and TVC counts are better than *Enterobacteriaceae* and TVB-N implying that are good indicators for making decision of freshness quality of chilled fillets stored at low temperatures (0-3°C).



Figure 27: Chilled fillets from establishments

4.4 PRESUMPTIVE SSOs

At the end of storage time for whole Nile perch and chilled fillets, few colonies from plates of IA and VRBGA were confirmed. Most colonies results were Gram-negative, rod shaped, oxidase positive, catalase positive, and Hugh and Leifson (O/F – fermentative in glucose). Few colonies were oxidase/catalase negative and non fermentative in glucose. The *Enterobacteriaceae* colonies from VRBGA also were Gram-negative, rod shaped, oxidase negative, catalase positive, and Hugh and Leifson (O/F – fermentative in glucose). These results imply that the main SSO (H₂S) on iced whole Nile perch and chilled fillets may be caused by *Shewanella putrefaciens*, *Pseudomonas*, *Vibrios/Aeromonas* and *Enterobacteriaceae* (figure 28).

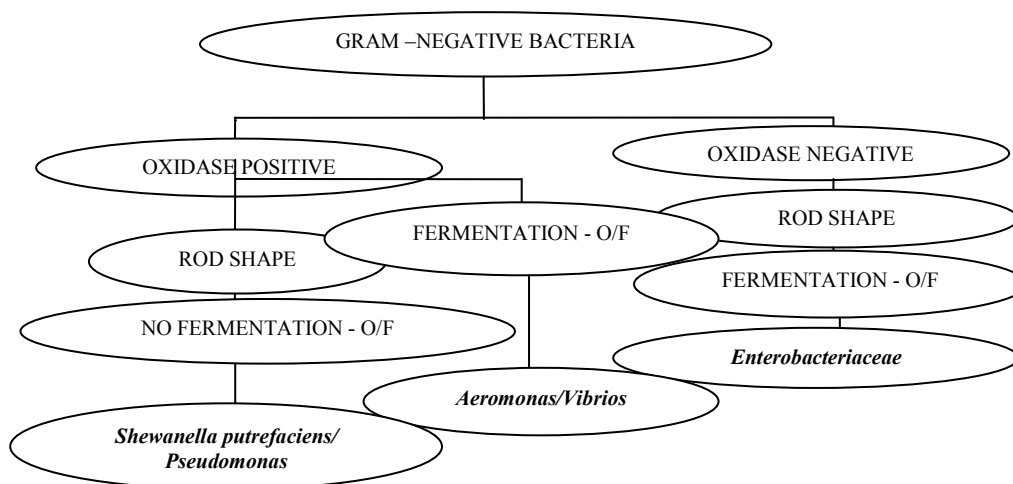


Figure 28: Flow chart employed for identification of Gram-negative specific spoilage bacteria in spoiling iced whole Nile perch and chilled fillets stored at 0-3°C.

4.5 SHELF LIFE OF ICED WHOLE NILE PERCH AND CHILLED FILLETS

The summary of results for estimated shelf life for iced whole Nile perch (both sensory assessment and microbiological counts) and chilled fillets (microbiological counts) stored at 0-3°C are shown in table 20. Shelf life of newly caught iced whole Nile perch was estimated to be about 4 weeks (26-28 days). The laboratory chilled fillets had a long shelf life (17 days) compared to establishments chilled fillets. The estimated shelf life for establishment's chilled fillets processed after long storage time of 17 days in ice was 8 storage days compared to 10-13 storage days for other chilled fillets (processed after 10, 6 and 5 storage days in ice).

Table 20: Shelf life (Estimated) and spoilage micro flora of iced whole Nile perch and chilled fillets stored at 0-3°C

TYPE OF SAMPLE	FISH SKIN	CHILLED FILLETS/ ICEDE NILE PERCH		EST. SHELF LIFE
LABORATORY/ ESTABLISHMENTS	LOGCFU/ CM ² /G	INITIAL-TVC LOGCFU/G	MAXIMUM-TVC LOGCFU/G	DAYS
4 DAYS (LAB)	4	4	10	17
5 DAYS (E4)	-	4	9	10-13
6 DAYS (E3)	5	3	10	13
10 DAYS (E1)	6	5	9	12
17 DAYS (E2)	5	5	10	8
NILE PERCH	4LOGCFU/G*	4*	11*	26-28*

*Results for newly caught whole Nile perch stored in ice

5 DISCUSSION

5.1 PHASE I: NATURAL MICROBIOLOGY OF WATER, SEDIMENTS AND WHOLE NILE PERCH

Bacteria in aquatic environment may be categorised into two groups which include those that are naturally present in the aquatic environment (indigenous bacteria) and others that could be present as a result of contamination by human or animal or bird's wastes or contamination during post-harvest handling and processing. The general results on microbiology of surface water, the bottom sediments and surface skin and guts of Nile perch in connection to the aquatic environment indicate different levels of microorganisms.

5.1.1 Phase I trial 1: Water

Low counts of microorganisms in water often indicate that the water is not polluted by animal manures or human wastes. On the other hand it implies that the water is not rich habitat of microorganisms because it is poor in nutritional content required by the habitats and growth of most microorganisms (Adams and Moss, 2008). Presence of *E. coli* is obviously indicating possibilities of recent contamination from animals or birds. While for presumptive *Vibrio cholerae* it could imply that *Vibrio* spp. is part of the indigenous micro flora of the aquatic environment where it is reported to occur naturally (Popovic *et al.*, 1993).

5.1.2 Phase I trial 2: Sediments

The significant total viable counts results on sediments may be taken into account that the sediments are natural reservoir of aquatic environmental (indigenous) micro flora that may contribute to the microbial load in living fish. Sediments may be the major source of contamination in catches/ fishes but it depends on method of fishing like trawling which may lead to high contamination, less with nets and least with long line methods. The low numbers of *Enterobacteriaceae*, *E. coli* and the absence of pathogenic microorganisms in sediments indicate that this family (*Enterobacteriaceae*) do not survive well in sediment as it is only dominant in the guts of animals such as fish. It also implies that there was no

contamination by human, animal or birds along Mwanza gulf fishing area. The presence of these indicators organisms and pathogens in sediments implies contamination from faeces of human or animal origin, (Huss, Dalgaard and Gram, 1997; Doyle, 1990).

5.1.3 Phase I trial 3: Whole Nile perch

The presence of *Salmonella* may indicate contamination by aquatic birds or from animal origin. *Salmonella* contamination of Whole Nile perch and Nile perch products can also result from fish handlers and contact surfaces if GMP/GHP/HACCP programmes are not properly adhered too. The results of this study show significant Total Viable Counts and *Enterobacteriaceae* counts in Nile perch guts samples. The Total Viable Counts range from $2.4 \times 10^2 - 1.0 \times 10^6$ cfu/g and the *Enterobacteriaceae* counts from $0 - 3.8 \times 10^4$ cfu/g. Both TVC and *Enterobacteriaceae* are in agreement with the range of 10^3-10^6 cfu/g of guts found by Adams and Moss (2008); Shewan (1962); ICMSF (1980b); and Liston *et al.*, (1976). Suspicious presence of *Vibrio cholerae* bacterium in one sample of Nile perch guts may be attributed to the feeding habit of fish which is carnivorous. It could have been feeding on other small pelagic fishes like sardines or small tilapias prior it was caught.

5.2 PHASE II: SHELF LIFE OF LABORATORY ICED WHOLE NILE PERCH AND CHILLED FILLETS

5.2.1 Phase II trial 1: Shelf life of iced whole Nile perch

5.2.1.1 Sensory/organoleptic assessment

The storage time of whole Nile perch was estimated 3- 4 weeks i.e. 26-28 days. The excellent and very good freshness quality fish scored < 10 and 10-12 total QIM scores respectively. This was in agreement to the main goal of QIM that enables processors to predict the remaining storage time of fish stored in ice (Larsen *et al.* 1992, Nielsen and Jessen 1997, Hydilg and Nielsen 1998, Martinsdottir *et al.* 2001). When the QIM score equals to 10 the remaining storage time in ice is estimated to be about 5 days (Huss, 1995). In this study, total QIM score was 10 when whole Nile perch storage time in ice was

between 15-20 days (2-3 weeks). Hence the estimated shelf life was likely to be 4 weeks or around 26-28 storage days as mentioned above. This is in agreement with Gram *et al.*, (1990) who reported long shelf life of Nile perch stored in ice of 28-30 days. The results of this study also show agreement with the study by Gram *et al.*, (1990) for Nile perch which was spoiled after 33 days storage time. The spoilage rate of tropical water fish in ice compared to temperate water fish is considerably slower as shown in a study by Gram *et al.*, (1987); and Ravn Jorgensen *et al.*, (1988). Their results demonstrated that the tropical water fish generally spoiled after 4 weeks compared to 9-10 days and rarely 2-3 weeks for temperate water fish.

5.2.1.2 Microbiological analysis

The microbiological parameters analysed on skin of whole Nile perch showed parallel and /correlated increase in counts throughout the storage time in ice. The TVC counts were the highest followed by SSOs counts and the lowest *Enterobacteriaceae* counts. The SSOs started with relatively low count but rapidly doubled (2- 6 log₁₀ cfu/g) compared to TVC (4- 6 log₁₀ cfu/g) within the first week of storage in ice. At the end of storage time (33 days) both SSO and TVC were > 10 log₁₀ cfu/g while *Enterobacteriaceae* was > 9 log₁₀ cfu/g. These counts of *Enterobacteriaceae* are high compared to 1.2 - 4.2 log₁₀ cfu/cm² reported in spoiling whole un-gutted rainbow trout (*Onchorynchus mykiss*) stored in ice for over 18 days storage period (Chytiri *et al.*, 2004) . Similar low counts were reported in different fresh Mediterranean product's shelf life (Ordonez *et al.* 2000; Tejada and Huidobro, 2002). In this work all analysed parameters reached between 8-9 log₁₀ cfu/g of skin of Nile perch after 17-22 days storage in ice. Similar counts of > 10⁸ cfu/cm² for SSOs were reported by Gram *et al.*, (1989, 1990) after 3 weeks storage time of whole Nile perch in ice. IMCSF, (1980b) reported TVC counts of nearly 10⁸ cfu/cm² that remained constant after 14 (>14) days of fish stored in ice. Gram *et al.*, (1990); Gram 1990; and Huss, (1995) reported that tropical water fish in ice has longer storage life than temperate water fish because of longer lag phase between 1-2 weeks for bacterial growth in tropical water fish compared to temperate fish.

In this study QIM (Organoleptic/sensory assessment) scores were far below the maximum score of 20-24 (table 5). The bacterial counts were really in high numbers beyond 8 log₁₀

cfu/g after 2-3 weeks storage in ice. In this study it has been noted that storage time up to 3 weeks (17-22 days in ice), freshness quality of whole Nile perch can be accepted to process good quality products (chilled fillets). The storage life can be extended to 28-30 days for products that can be frozen or heat treated. At the end of storage time after 33 days the microbial loads remained constant at $\geq 10 \log_{10}$ cfu/g when the fish was judged spoiled. The bacterial load on skin is usually lowered by washing fish (with treated water) during processing at establishments and by low temperature treatment like chilling/super chilling and freezing of processed fillets/products. In this context it is important to note that microbial loads in freshly caught fish may range from hundreds to millions (10^2 - 10^7) per square centimetres on skin depending on species and or fishing areas (Adams and Moss (2008); Shewan (1962); ICMSF (1980b); and Liston *et al.*, 1976).

5.2.1.3 Chemical analysis

Total volatile base nitrogen changes during storage of whole Nile perch in ice was characterized with a small margin of 6-8mgN/100g of fish flesh in the first 3 weeks of storage in ice but then increased to 17mgN/100g at the end of storage after 33 days. This indicates that TVB-N is not a good indicator for the changes in freshness quality of whole Nile perch as the QIM scores and spoilage micro flora counts. It was reported by Gram *et al.*, (1989), that TVB-N in Nile perch which hardly reached ≥ 14 -24mgN/100g. Similar low levels of TVB-N approximate 14-20mgN/100g were reported on whole un-gutted rainbow trout fresh water fish (Chytiri *et al.*, 2004) and on European sea bass (*Decentrarchus labrax*) by Castro *et al.*, (2006). In accordance with ICMSF, (1980b) these levels are very low compared to other species which may have TVB-N in the range of 20-30mgN/100g during the first 2 weeks and > 30 mg/100g after 2 weeks of storage in ice. Other authors have reported TVB-N values between 25mgN/100g (Castro *et al.*, 2006) and 30-35mgN/100g (Connel, 1995; Huss, 1988) in European sea bass and rainbow trout (Gimenez *et al.*, 2002) stored in ice. Thus in this study the TVB-N value in Nile perch was well below the maximum limits 30-35mgN/100g and 25mgN/100g mentioned before. A noticed change in TVB-N occurred when the SSOs counts reached $> 9 \log_{10}$ cfu/g which is in agreement with observation made by Dalgaard *et al.*, (1993), who reported that counts 10^8 - 10^9 cfu/g of *S. putrefaens* (SSO) are required for production of TMA. The TMA is one of

the components contributing to TVB-N content. The value of TVB-N at which Nile perch is unacceptable is not well established, Karungi *et al.*, (2004).

The change/shift in pH units was constant (7.01-7.11) throughout storage time in ice. This was higher than the pH reported by Gram *et al.*, (1989) which was 6.7-6.8 units but same small shift during storage i.e. within 0.1 units. Constant small shift has also been reported on rainbow trout (Chytiri *et al.*, 2004) and European sea bass, (Castro *et al.*, 2006).

5.2.2 Phase II trial 2: Shelf life of laboratory chilled fillets

5.2.2.1 Microbiological analysis

Storage time of Laboratory chilled Nile perch stored at 0-3°C was between 17-23 days. Shelf life for good quality on freshness chilled fillets was 17 storage days. The average counts for both SSO and TVC were between 6-8 log₁₀ cfu/g. Study on chilled Nile perch fillets stored at 0°C by Gram *et al.*, (1989) found total viable counts of 5 x 10⁷cfu/g and SSO counts of 10⁴-10⁵cfu/g at the end of storage time (3 weeks). In the same study on contaminated fillets with *Pseudomonas*, the SSO counts were 10⁸-10⁹cfu/g in 10 days of storage time and remained constant for the rest of storage time which is in agreement with the present study. In this study the counts were stable from 20-23 days of storage. Counts of *Enterobacteriaceae* were constant around 4 log₁₀ cfu/g during the first 17 days of storage but then increased to a stable 8 log₁₀ cfu/g at day 18 until end of storage time. The growth rate of *Enterobacteriaceae* may be slower at low temperatures (0-3°C) than that of other Gram-negative psychrotrophic spoilers (Chytiri *et al.*, 2004). Mesophilic organisms like *Enterobacteriaceae*; require conditioning during chill storage temperature before they can grow in big numbers. On the basis of this study *Enterobacteriaceae* may be considered as a part of the spoilage micro flora in chilled Nile perch fillets. Likewise, Hozbor *et al.*, (2006) and Fønnesbech *et al.*, (2005) reported *Enterobacteriaceae* in fresh salmon ice at aerobic storage conditions.

5.2.2.2 Chemical analysis

The level of Total volatile base nitrogen (TVB-N) in chilled Nile perch fillets was constant at 6-7mgN/100g for the first 12 days of storage but increased rapidly after that to a value of 27mgN/100g at the end of storage period of 23 days. This narrow change was also observed in whole Nile perch for the first 3 weeks. However the maximum TVB-N reached in chilled Nile perch 27mgN/100g was higher than the level on whole Nile perch which was 16mgN/100g. Similar levels of TVB-N values of 18-26mgN/100g were reported on rainbow trout fillets (Chytiri *et al.*, 2004). This difference in TVB-N between whole Nile perch and chilled fillets may be due to low numbers of spoilage bacteria penetration and growth in the un-exposed flesh of whole Nile perch as compared to exposed chilled fillets (ICMSF, 1986). Rapid increase in TVB-N corresponded with high counts ($> 8 \log_{10}$ cfu/g) of SSO and TVC in chilled fillets while similar changes corresponded with $> 9 \log_{10}$ cfu/g of the same counts in the skin of whole Nile perch. This is in agreement with Chytiri *et al.*, (2004) results in similar study on rainbow trout fillets stored in ice. In the present study the results are much lower than those earlier reported TVB-N of 48-60mgN/100g in Nile perch fillets stored at ambient temperature (Karnick and Lima dos Santos, 1985) and the limit of acceptability for iced stored cold water fish 30-35mgN/100g (Connell, 1995 and Huss, 1988). The changes in concentration of TVB-N in spoiling fish has been mentioned as unreliable indicator for fish freshness, (Chytiri, *et al.*, 2004; Dawood *et al.*, 1986; Kyrana *et al.*, 1997; Castro *et al.*, 2006 and Tejada and Huidobro, 2002). The change in pH on chilled Nile perch fillets was within the neutral pH range 6.4 to 7.0 units during the storage of whole Nile perch above.

5.3 PHASE III: MICROBIOLOGY OF WHOLE NILE PERCH SWABS, ON LINE FILLETS AND SHELF LIFE OF ESTABLISHMENTS CHILLED FILLETS

5.3.1 Phase III - Trial 1: Microbiology of whole Nile perch swabs and online fillets

Swabs which were collected in three different establishments from whole Nile perch stored for long and short times (days) in ice (for 10 days - E1, 17 days - E2, and 6 days - E3 days) prior to processing. Counts prior to wash for *Enterobacteriaceae* and TVC were 2-3 \log_{10} cfu/cm² and 5-6 \log_{10} cfu/cm² respectively. Irrespective of the difference in storage days in

ice there was only a small margin (different) of about $1 \log_{10} \text{ cfu/cm}^2$ in both parameters on the skin prior to wash between the three factories (establishments). The levels of TVC counts reflect that whole fish was stored in ice for considerably time. In accordance with ICMSF, (1986), normal micro flora counts on the skin of fish at the time of harvest are in the region of $10^2 - 10^5$ organisms per gram while an increase to levels of $> 10^6$ per gram indicates long storage in ice (chill conditions). During storage in ice, the bacteria loads, blood and slimes on skin are continuously washed away by melting ice. The Total viable counts (prior-wash) in this study are within the range reported by Adams and Moss (2008); Shewan (1962); ICMSF (1980b); and Liston *et al.*, (1976); that, the bacterial loads on the skin of fish from catch can range from hundreds up to millions per square centimetres (10^2 - 10^7 numbers/cm²) of skin. Gibson and Ogden, 1987; and Dalgaard and Huss, 1994), showed that the slow increase in counts may be caused by effect of ice where by it retards the growth of microorganisms to less than one-tenth of the rate at optimal growth rates. While compared with the relative growth rate of microorganisms at 20-30°C which is approximately 25 times higher than at 0°C. The effect of washing whole fish on reducing bacterial loads was more significant for *Enterobacteriaceae* than for total viable counts. There were more than $1 \log_{10} \text{ cfu/cm}^2$ decreases after wash in *Enterobacteriaceae* at all three establishments but only in one establishment in TVC counts. Hence these results reveal a good bacteriological freshness quality of whole fish for processing as the counts were within the limits referred to previously.

The results prior and after washing for online fillets corresponding to the above different fish lots and establishments show some difference between establishments. Counts prior to fillets washing were 2,91 – 4,55 $\log_{10} \text{ cfu/g}$ (*Enterobacteriaceae*) and 5,49 – 6,27 $\log_{10} \text{ cfu/g}$ (TVC). A guideline on TVC level for fresh fish and fish products microbiological quality including fillets is 5×10^5 - 10^7 colony forming units per gram (cfu/g) of muscle (ICMSF, 1986) and for *Enterobacteriaceae* $\leq 10^3 \text{ cfu/g}$ of muscles (fillets). According to the results before washing, the counts in 2 establishments were above levels of 10^3 cfu/g and in all 3 establishments above $5 \times 10^5 \text{ cfu/g}$ for *Enterobacteriaceae* and TVC respectively. This is indicating a likely contamination and or growth of microorganisms during the subsequent processing steps at establishments. However after wash the microbiological loads for E2 and E3 in fillets were significantly reduced below the maximum allowable limits $< 10^3 \text{ cfu/g}$ (*Ent.*) and $< 5 \times 10^5 \text{ cfu/g}$ (TVC). There was no significant reduction in microbial load in E1 which was realized that both (two) samples were by mistake taken before wash. Generally this may imply that

washing stage for fillets prior chilling/freezing is paramount important in order to reduce microbiological loads.

5.3.2 Phase III – Trial 2: Shelf life of establishments chilled Nile perch fillets

The estimates on storage time for chilled Nile perch fillets processed after long and short storage (10, 17, 6, & 5 days) of whole fresh Nile perch in ice from four establishments, E1-E4 respectively was approximately 12, 8, and 10-13 days when the SSO/TVC counts reached 6-8 log₁₀ cfu/g. The microbiological counts in long time (17 days) ice stored fish fillets were above the ICMSF-FAO/EU maximum guidelines/limits (7 log₁₀ cfu/g) after 8 storage days compared to the short time (5 days) ice stored fish which reached these counts after 10 -13 days of storage (0-3°C). The rate of spoilage has been reported being slow from skin and increases for gutted, fillets and finally minced fish stored at chill conditions (ICMSF, 1980b). On the other hand long time storage of fish in ice allows growth of psychrotrophic bacteria (SSO) to big numbers, ICMSF, (1986) which definitely reduce the shelf life of final processed fillets.

Many studies have been published for the range of TVB-N values in Nile perch (Karungi *et al.*, 2004). Many of these studies reported a concern that this parameter is not good indicator for fish products freshness at ice/chill storage as in 5.2.2.2 laboratory chilled fillets above. This was also reported by Rehbein and Olchenschlager, (1982); Chytiri *et al.*, (2004); Castro *et al.*, 2006. The total volatile base amine (TVB-N) produced during later/advanced stages on chill/ice stored fish. Several authors have reported TVB-N value of ≤ 25mgN/100g for rainbow trout, European sea bass and fresh Mediterranean fish (Chytiri *et al.*, (2004); Castro *et al.*, (2006); Kyrara and Lougovois, (2002) and Gimenez *et al.*, (2002).

5.3.2.1 Establishment E1 (10 days iced whole Nile perch)

The storage life of fillets was approximately 12 days prior the beginning of a rapid log phase with rapid increase in the numbers of SSO, TVC and *Enterobacteriaceae* from 12-15 storage days. It seems that the increase in number of psychrotrophic bacteria (SSO, TVC) is relatively slow in the first 2 weeks at chill storage (0-3°C). In this trial a common trend was observed to the laboratory chilled fillets trial in that both studies gave similar counts

(around 6 log₁₀ cfu/g) after 12 storage days regardless of the longer storage time of whole Nile perch in ice prior processing at establishments. The long shelf life of fillets with initial high counts of > 4 log₁₀ cfu/g can be explained by the lower psychrophilic counts in tropical/fresh water fish compared to temperate water fish (Disney (1976) and Shwewan (1977)).

Total volatile base nitrogen changes were small and parallel with the *Enterobacteriaceae* counts during the first 12 days of storage. The increase in TVB-N levels depend on numbers of SSO, H₂S (black colonies) producing bacteria i.e. *Aeromonas* and *Shewanella* (Gram *et al.*, 1990). Likewise *Enterobacteriaceae* and non H₂S (white colonies) producing bacteria which include *Pseudomonas*, *Alcaligenes*, *Moraxella*, and *Acinetobacter* can also produce TVB-N (Gram *et al.*, 1987). In all spoilage micro flora and chemical changes were rapid after 12 days of storage. This means bacterial activity in the fish flesh leads to accumulation of TVB-N. The TVB-N content include TMA which is produced by bacteria when they are at higher numbers i.e. $\geq 10^8$ - 10^9 cfu/g (Chytiri *et al.*, 2004; Rehbein and Oehbeinschlager, 1982).

5.3.2.2 Establishment E2 (17 days iced whole Nile perch)

Estimated storage life for the fillets based on the spoilage microbiological (SSO and TVC) counts is 8 days. This brings into attention that Nile perch fillets that are aimed to be stored for a long time i.e. (>10 days) should be processed from a fish stored in ice for a maximum of 10-14 days. In Establishment E2 the whole fish had been stored in ice for 17 days prior to processing. Here the fillets showed a spontaneously log phase from the beginning of storage to the end with highest counts up to > 10 log₁₀ cfu/g which has rarely been reported. The ICMSF, (1980b), reported four stages of fresh fish when stored in ice (table 4). At stage IV (> 14 storage days in ice) microbiological counts may reach $\geq 10^8$ cfu/cm² and remain constant during the remaining storage time. The same was observed in the present work and also in a study by Gram *et al.*, (1989). Although super chilling treatment may have arrested or killed most of the microbial load there are still many which have survived and can account for the fast multiplication to levels beyond 10 log₁₀ cfu/g within 2 weeks of storage. This period (2 weeks) is declared by processors as the maximum shelf life/storage time for chilled fillets. However, it is important to fulfill the markets/ consumers requirements with respect to set shelf life as well as means of transportation and storage

conditions which will maintain the cold chain. The shelf life can be extended if storage is at super chilling conditions $< 0^{\circ}\text{C}$ unlike the $0-3^{\circ}\text{C}$ used for this trial. Storage at sub zero temperatures (-2 and -3°C) is reported to increase shelf life (Riaz-Fatima *et al.*, 1988 and Sivertisvik *et al.*, 2003; Huss 1995; Chang *et al.*, 1998).

The TVB-N changes were parallel to changes in the spoilage micro flora above. However the TVB-N changed from approximate $7-11\text{mgN}/100\text{g}$ which is well below the maximum acceptability to temperate water fish of $30-35\text{mgN}/100\text{g}$, (Rehbein and Olchlenschlager, 1982; Connel, 1995; Huss, 1988).

5.3.2.3 Establishment E3 (6 days iced whole Nile perch)

The estimated storage life was 13 days. It is interesting that these results show shelf life that is close to that of the fillets from the whole fish that was stored longer in ice or for 10 days. The spoilage flora was characterised by very short lag phase and dominated by a log phase for 2-13 storage days and resulted in a stationary phase ($\geq 8-9 \log_{10} \text{cfu/g}$) after 13-17 storage days. Similar results was reported by Gram *et al.* (1989) in contaminated (*Pseudomonas*) Nile perch fillets with counts from 10^8-10^9cfu/g after 10 storage days that remained constant during the rest of the storage time. Refer also to 5.2.2.1 and 5.3.2 above.

Remarkably the TVB-N changes remained constant for the first 13 storage days ($6\text{mgN}/100\text{g}$) and finally increased to approximately $11\text{mgN}/100\text{g}$ at the end of storage. As previously the TVB-N (5.3.1.3 above) is low compared to the maximum limits $30-35\text{mgN}/100\text{g}$. Accordingly it was not good quality indicator for fillets during storage at chill conditions, see 5.2.2 .2 and 5.3.2 above. The same can be said for the pH shift from 6.8 to 7.3 units.

5.3.2.4 Establishment E4 (5 days iced whole Nile perch)

Storage time was estimated approximately 10-13 days. Changes in both spoilage micro flora and chemical TVB-N gave similar trends as mentioned before in 5.2.2.2. The TVB-N slowly increased from $5-13\text{mgN}/100\text{g}$ as described in 5.3.2 above.

5.4 PRESUMPTIVE SSO

There are two types of colonies-counts which are reported from growth on Iron Agar (IA), SSO H₂S producers (black colonies) that may include bacteria like *Shewanella putrefaciens* and *Vibrionaceae/ Aeromonas* and the SSO non H₂S producers (white colonies) that include *Pseudomonas spp* as well as *Enterobacteriaceae spp* (figure 34). These SSOs were identified by Gram *et al.*, (1989) in whole Nile perch and fillets stored at 0°C. Similar studies by Chytiri *et al.*, (2004) in rainbow trout; Fonnesbech *et al.*, (2005) and Hozbor *et al.*, (2006) in sea salmon all identified similar SSOs as Gram-negative, rod shaped, oxidase positive and glucose fermentative/non fermentative and or H₂S producers and non H₂S producers in IA.

6 CONCLUSIONS AND RECOMMENDATION

6.1 CONCLUSIONS

Microbiology of fishing grounds in Mwanza gulf showed remarkable bacteria counts in sediments and whole Nile perch guts while lake water counts were approximately to detection limits i.e. 0-20cfu/ml. The human pathogen *Salmonella* and *Vibrios spp* were only detected in 2 samples out of 20 tested, one sample of lake water was positive for *V. cholerae* and one sample of Nile perch was positive for *Salmonella*. Therefore it may be concluded that these human pathogens occasionally may be derived from the fishing environments of Nile perch.

The average keeping quality (shelf life) in days for iced whole Nile perch, laboratory and establishments chilled fillets were 26-28; 17; 8-13 days respectively. Shelf life was short (8 days) for long time (17 days) stored whole Nile perch in ice before processing. Hence it may be suggested that the preferably maximum storage time for freshness good quality whole Nile perch in ice is 1-2 weeks. While for longer storage periods of approximately of 2-3 weeks the fish can be processed for other products like frozen than chilled fillets respectively. In this work, the main spoilage organism's composition in spoiled whole Nile perch and chilled fillets could include *Shewanella putrefaciens*, *Vibrionaceae/ Aeromonas*, *Pseudomonas*, and *Enterobacteriaceae*.

Both sensory/organoleptic (QIM score) assessment and microbiological methods were consistent/good while the chemical method was not good enough during the early storage for whole Nile perch in iced and chilled fillets. Hence the chemical (TVB-N) parameter is not suggested as a reliable indicator for freshness quality of iced whole Nile perch as well as chilled fillets.

6.2 RECOMMENDATION

1. Similar shelf life study is recommended to be repeated at different storage temperatures such as at sub zero (-1 to +1°C); 3-5°C; 6-8°C and 20-24°C. Shelf life studies in other commercial fishes like Nile tilapia and Cat fish are also recommended.
2. Fish establishments should ensure that chilled fillets are only processed from short time iced whole Nile perch i.e. 1-2 weeks.
3. To establish appropriate quality index measurement (QIM) for whole Nile perch freshness sensory/organoleptic assessment.
4. More characterization for main SSOs possibly to a species level is required. To enable closer identification of the specific spoilage organisms including *Pseudomonas spp.*, *Vibrio/Aeromonas spp.*, *S. putrefaciens*, *Enterobacteriaceae* and others.
5. To extend study on food safety microorganisms in fish and environment of Lake Victoria is highly recommended.
6. To establish maximum limits of acceptance for freshness quality chemical parameters including TVB-N and others in Nile perch products.
7. To establish guidelines on bacterial count limits for ascertaining remaining storage time or acceptability of raw fish for processing of various fish products.

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APPENDICES

Appendix I: Freshness ratings using the quality assessment scheme used to identify the quality index demerit score (Larsen *et al.* 1992).

Quality parameter	Character	Score (ice/seawater)			
		0	1	2	3
General appearance	Skin	Bright, shining	Bright	Dull	
	Bloodspot on gill cover	None	Small, 10-30%	Big, 30-50%	Very big, 50-100%
	Stiffness	Stiff, in <i>rigor mortis</i>	Elastic	Firm	Soft
	Belly	Firm	Soft	Belly burst	
	Smell	Fresh, seaweed/metallic	Neutral	Musty/sour	Stale meat/rancid
Eyes	Clarity	Clear	Cloudy		
	Shape	Normal	Plain	Sunken	
Gills	Colour	Characteristic, red	Faded, discoloured		
	Smell	Fresh, seaweed/metallic, rancid	Neutral	Sweaty/slightly rancid	Sour stink/stale
Sum of scores	minimum 0 and maximum 20				

Appendix II: Iron agar (IA) for total viable counts (psychrotrophic counts) including detection of black colonies (H₂S), Gram et al., (1987).

Ingredients/Composition

1. Peptone 20g
2. Lab lemco powder 3g
3. Yeast extract 3g
4. Ferric citrate 0.3g
5. Sodium thiosulphate 0.3g
6. Sodium chloride 5g (10g for *P. Phosphoreum*)
7. Agar 15g
8. 990ml distilled water

Sterilize at 121⁰C/15minutes and add 10ml (4%) filtrate, L-cysteine is added after sterization - (5.8g cysteine –HCL/100ml water)

Appendix III: Results of iced whole Nile perch and chilled fillets

A. Results of QIM scores in iced whole Nile perch

		DAYS	4	7	10	13	15	20	22	26	33
GENERAL APPEARANCE (GA)	GA SKIN		0,14	0,67	0,60	0,95	0,90	1,29	1,54	1,50	1,67
	GA BS-GILLS		0,14	0,39	0,00	0,71	0,52	1,30	0,80	1,17	1,13
	GA STIFFNESS		0,81	0,83	0,73	1,05	1,14	1,74	1,00	1,33	2,50
	GA BELLY		0,52	0,39	0,20	0,52	0,86	1,31	0,61	1,00	1,08
	GA SMELL		0,19	0,56	0,93	1,05	0,86	1,74	1,80	1,33	2,13
EYES (E)	E CLARITY		0,10	0,44	0,40	0,57	0,57	0,93	0,69	1,00	1,00
	E SHAPE		0,00	0,06	0,07	0,24	0,33	1,59	0,99	1,83	1,71
GILLS	GILL COLOUR		0,05	0,17	0,20	0,52	0,52	0,94	1,00	1,00	1,00
	GILLS SMELL		0,19	0,61	0,93	1,19	1,24	2,00	1,93	1,83	2,21
QIM	QIM SCORES		2,14	4,11	4,07	6,81	6,95	12,86	10,36	12,00	14,42

B. Results of shelf life on iced whole Nile perch

		DAYS	4	7	10	13	15	17	20	22	26	33
SPOILAGE MICRO FLORA	SSO (H ₂ S)											
	logcfu/g		2,75	6,20	6,25	6,53	6,15	8,38	9,29	9,49	10,54	10,55
	TVC logcfu/g		4,12	6,58	6,67	7,40	7,88	8,70	9,52	9,84	11,01	10,91
	Ent. logcfu/g		4,40	5,67	5,64	6,22	5,64	6,55	8,22	8,04	8,23	9,51
QIM	SCORES		2,14	4,11	4,07	6,81	6,95	-	12,86	10,36	12,00	14,42
TVB	mgN/100g		7,48	7,25	6,51	6,93	7,23	7,52	6,93	8,60	11,99	16,80

C. Results of shelf life for Laboratory chilled fillets

SPOILAGE MICRO FLORA	DAYS	2	6	10	12	17	19	21	23
	SSO (H ₂ S)								
logcfu/g		1,67	5,72	4,93	6,25	8,35	8,74	9,31	9,31
TVC									
logcfu/g		4,42	6,38	6,43	7,43	8,54	9,61	9,67	9,67
<i>Enterobacteriaceae</i>									
Ent.logcfu/g		4,60	3,31	3,70	3,52	4,50	8,09	8,07	8,07
TVB	mg N /100g	6,72	7,67	7,50	7,67	13,90	14,65	17,07	26,92

D. Results of shelf life for Establishments (E1 & E2) chilled fillets

SPOILAGE MICRO FLORA	ESTABLISHMENT E1 (10 Days in ice)					ESTABLISHMENT E2 (17 Days in ice)			
	DAYS	2	8	12	15	2	8	12	15
SSO (H ₂ S) logcfu/g		4,30	6,38	6,38	8,24	4,02	7,05	8,64	10,04
TVC logcfu/g		5,39	6,98	6,98	9,38	4,71	7,51	9,12	10,38
Ent.logcfu/g		3,00	3,34	3,34	7,51	2,15	2,30	3,24	4,43
TVB	mgN/100g	8,36	6,55	8,65	21,09	5,65	7,56	7,52	10,63

E. Results of shelf life on Establishments (E3 & E4) chilled fillets

SPOILAGE MICRO FLORA	ESTABLISHMENT E3 (6 Days in ice)						ESTABLISHMENT E4 (5 Days in ice)					
	DAYS	2	6	10	13	15	17	2	6	10	13	15
SSO (H ₂ S)												
logcfu/g		3,00	4,02	6,18	7,91	8,46	8,42	3,42	4,31	7,03	8,69	8,98
TVC logcfu/g		3,20	4,78	7,40	8,52	9,24	9,53	3,58	5,13	7,31	9,05	9,48
Ent.logcfu/g		2,17	2,00	2,00	3,24	-	-	2,83	2,50	2,50	3,66	-
TVB	mgN/100g	5,86	5,67	6,55	5,63	7,48	10,71	4,96	4,96	7,18	8,02	13,32