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DEVELOPMENT OF A QUALITY INDEX METHOD (QIM) SCHEME FOR FARMED SENEGALESE SOLE (*Solea senegalensis*) AND ITS APPLICATION IN A SHELF LIFE STUDY

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ABSTRACT

Temperature is one of the most important factors determining the shelf life of fish. Sensory evaluation is the most important method to assess the freshness quality of seafood. The Quality Index Method (QIM) is a sensory method that can be applied to evaluate fish freshness. The objective of the project was to develop QIM for Senegalese sole and to study the influence of temperature abuse on the freshness and quality of Senegalese sole in a shelf life study. During the shelf life study, one group of fresh farmed Senegalese sole was stored in ice for 16 days (Control group) and a second group was stored at room temperature (20°C) for 16 hours followed by iced storage up to 16 days (T-abused group). Quality changes during storage were observed with sensory evaluation using the Quality Index Method (QIM), Torry freshness scoresheet for cooked fatty fish, chemical and microbial analysis of total viable counts (TVC), hydrogen sulfide (H_2S) producing bacteria, and total volatile basic nitrogen (TVB-N). The QIM developed for whole fresh Senegalese sole consisted of 10 quality parameters, and different freshness categories, resulting in a total of 26 demerit points. A high correlation between QI and storage time in ice was observed (R^2 = 0,9816). A shelf life of 12-16 days was observed for both groups (Control and T-abused), based on sensory evaluation of cooked Senegalese sole. After 16 days of storage, Total viable microbial counts had reached 7 log10 cfu/g in both groups. The H_2S – producing bacteria values were higher in T-abused group, 6 log10 cfu/g compared to 5 log 10 cfu/g in the Control group. Low levels of TVB-N were found during the storage time, ranging between 16.3 and 18.05 mgN/100g.

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ACRONYMS AND ABBREVIATIONS

CA	Competent Authority
DMA	dimethylamine
EC	European Commission
EEZ	Exclusive Economic Zones
EU	European Union
FA	formaldehyde
FP	Fish Products
FVO	Food and Veterinary Office
GoSL	Government of Sierra Leone
GDA	Generic Descriptive Analysis
HACCP	Hazard Analysis and Critical Control Points
IEZ	Inshore Exclusive Zones
IUU	Illegal, Unreported, Unregulated
Kg	Kilogram
Km	Kilometer
MDA’s	Ministries, Departments and Agencies
MFMR	Ministry of Fisheries and Marine Resources
MoHS	Ministry of Health and Sanitation
MWR	Ministry of Water Resources
Mt.	Metric Tonnes
PCBs	Polychlorinated biphenyls
SLSB	Sierra Leone Standards Bureau
SSOs	Specific Spoilage Organisms
Sq.	Square
t	Tonnes
TMA	Trimethylamine
TMAO	Trimethylamine N-oxide
TVB-N	Total Volatile Basic Nitrogen
TVC	Total Viable Counts
TVN	Total Volatile Nitrogen
QI	Quality Index
QIM	Quality Index Method

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1 INTRODUCTION

Sierra Leone has a tropical climate and lies between latitudes 7°N and 10°N, and between longitudes 10° and 13°W, on the coast of West Africa with diverse vegetation ranging from savannah and grassland to tropical rainforest. It is bordered on the Southwest by the Atlantic Ocean, North and Northeast by the Republic of Guinea and to the Southeast by Liberia (Figure 1). Sierra Leone covers a total area of about 71,740 km^2 with a coastline of about 560 km (MFMR, 2008). Sierra Leone lies north of the Gulf of Guinea, and at the intersection of the Canary Current in the north and the Guinea Current in the south. One of the important factors influencing the productivity of Sierra Leonean waters and the extent of fishing activities is water movement. Sierra Leone has a continental shelf which combined with the local currents creates a substantial upwelling that places the country within one of the world's most productive marine ecosystems (Heyman & Vakily, 2004). Sierra Leone has an estimated population of c.a. 7 million (Koroma, 2015). The continental shelf of Sierra Leone is about 100 km wide in the north and tapers to about 13 km in the South towards Liberia. The total continental shelf area covers about 30,000 km^2. The area of the 200 miles Exclusive Economic Zone (EEZ) is about 15,700 km^2 and most of the fishing activities take place within 17,000 km^2 of the Exclusive Economic Zone (MFMR, 2003).

Figure 1: Map of Sierra Leone

Fish is an important source of animal protein and the most affordable protein source for the majority of people in Sierra Leone. Sierra Leone's marine fisheries are unevenly exploited. Some fishing grounds are under heavy pressure with levels of fishing effort greater than the maximum sustainable yield. Though fish has become the readily available source of animal protein, poor handling and processing result in lower prices or sometimes large quantities of fish are discarded (Getu & Misganaw, 2015).

In the fishery sector, post-harvest fish losses and seafood contamination are of great concern because they result in loss of income and present a health risk to consumers. This is largely due to obsolete processing practices and poor technology induced by lack of capacity. The success of any effort in the fishery industry is highly dependent on getting the product to the consumer in an acceptable condition. Therefore, efforts are required to maintain the quality and acceptability of the fish and fisheries products from harvest to the consumers (Getu & Misganaw, 2015).

Most of the fish caught by small-scale fishermen in Sierra Leone is sold soon after landing either for human consumption or for further processing. This catch is often in a poor condition when landed, either as a result of exposure to high temperatures during storage and transportation to the landing site or physical damage due to poor handling and processing techniques. The impact of good handling on board fishing boats and the quality of their catch is quickly lost due to the quantity of ice taken aboard when the fish is unloaded and put on the sandy beach or on plastic sheets (Green, Carrol, & Mason, 2012).

Maintaining good sensory evaluation as a tool for evaluating fish freshness and quality is important. From the moment the fish is caught the deterioration process starts and quality of the fish is affected. The quality of fish is strongly influenced by storage temperature as temperature affects bacterial growth and autolysis (Andrade et al., 2015).

Farmed fresh Senegalese sole (Solea senegalensis) was chosen as a sample for the study due to its similarity to the sole (Cynoglossus senegalensis) which is one of the important fish species for export in Sierra Leone.

It is hoped that knowledge gained from this research will contribute to monitoring of fish standards and improved fish handling and processing methods in Sierra Leone thereby making the products safe and of higher overall quality; as well as mapping out procedures for training of inspectors on sensory evaluation of fish freshness. It is also hoped that this study will contribute to the management of fisheries in terms of decision making on policies and laws governing the fisheries, especially in relation to fish safety and quality assurance.

The overall aim of the project is to improve the quality and value of the fish catch through processing in Sierra Leone. This would be achieved by an understanding of the influence of temperature on fish during the early stages of storage and its effect on the shelf life of the product. Further, the aim is to propose formative ways of addressing the challenges facing the freshness of fish with respect to sensory quality by being able to develop sensory methods of evaluating fish freshness, such as the Quality Index Method.

The objectives of this study are:

- To map out the procedures of designing sensory methods for fish freshness, such as the Quality Index Method. The Quality Index Method is based on species specific quality changes with storage time.
- Study the effects of different temperatures (0°C during the whole storage time compared to real conditions of 20°C for 16 hours before cooling to 0°C).
- To identify the major causes of fish freshness deterioration and to identify and suggest ways to improve the quality and value of fish.

2 THE SIERRA LEONEAN FISHERIES

The maritime zones of Sierra Leone are classified into inshore and offshore zones. The inshore zone is exclusively reserved for artisanal fisheries operations and is extended up to 6 nautical miles from the baseline and beyond the Exclusive Economic Zone (EEZ), which is the area of operations of the industrial fishing fleet. The marine fisheries of Sierra Leone play an important role in the economy of the country, supporting the livelihoods of many coastal communities as well as contributing significantly to food security. About 200 different marine species have been identified and recorded in the fishery sector and about 100 are commercially valuable species (Jalloh, 2010). Data collection is well established in the policy of the Ministry of Fisheries and Marine Resources, but this is not maintained and reviewed regularly. The marine artisanal and industrial fisheries exploit the marine resources of Sierra Leone. An estimated biomass of approximately 102,684 metric tonnes in 2017 was exploited by the industrial fisheries. However, these do not account for Illegal, Unreported, and Unregulated (IUU) catches. The marine artisanal fishery is based primarily on small pelagics constituting about 60% (MFMR, 2018) of the total landings.

The fish resources of Sierra Leone may be classified into four main categories: (i) demersal species mostly the Sciaenidae – e.g. the croakers (*Pseudotolithus senegalensis*); (ii) pelagics dominated by the clupeids (*Ethmalosa fimbriata*, *Sardinella maderensis*); (iii) crustaceans (shrimps, crabs and Lobster); (iv) Cephalopods (cuttlefish) and Molluscs (octopus, squids and snails). The fishery of Sierra Leone is broadly divided into artisanal fisheries, industrial fisheries, and inland fisheries and aquaculture.

2.1.1 The Artisanal Fisheries

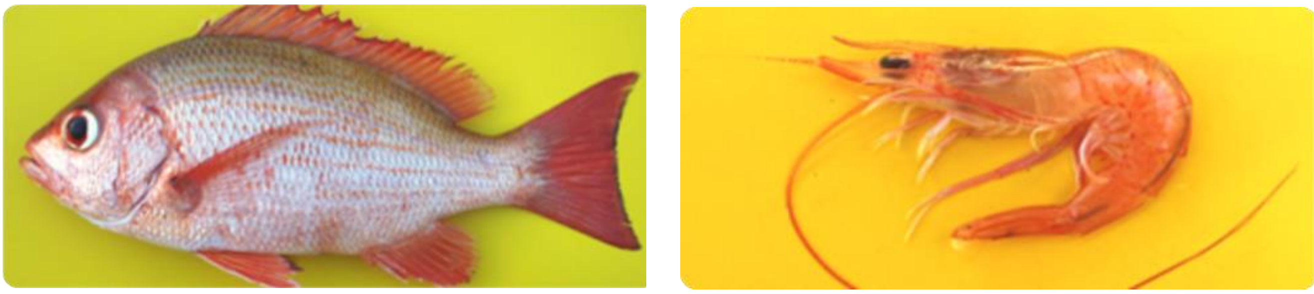
The artisanal fisheries sector which is a low technology small-scale fishery operates from over 600 fish landing sites along the six major coastal districts of Sierra Leone. This sector consists of a variety of fishing gears such as long liners, cast nets, purse seiners, ring nets, beach seiners, traps and hooks operating from different fishing boats. Most of the boats are equipped with an outboard engine and fish boxes. Most boats take ice on board to preserve their catch. The marine resources, particularly the fishery is a common property resource and remains an open access fishery (Jalloh, 2010). It creates direct employment for about 40,000 fishermen and an estimated 500,000 additional jobs are provided by ancillary activities like fish processing, marketing or boat-building/repairs (Koroma, 2015). Women dominate the value chain in the artisanal sector: fish processing, distribution and marketing. The total annual production in the artisanal sector is approximately 120,000 metric tonnes and thus contributing to food for the poor fishing communities. Potential for increased fish production in this sector exists but this requires major investment in technology, fish handling, processing and data collection and analyses (MFMR, 2018). About 80% of all fish consumed by Sierra Leoneans is caught by the artisanal fisheries sector. The dominant species in the artisanal sector are the clupeid (*Sardinella maderensis* and *Ethmalosa fimbriata)* (Figure 2) which constitute about 60% of their total landings and 30% of the catch are juveniles (Jalloh, 2010).

a. *Ethmalosa fimbriata* (Bonga) b. *Sardinella maderensis* (Flat herring)

Figure 2: Bonga - *Ethmalosa fimbriata* (a) and Flat herring - *Sardinella maderensis* (b)

2.1.2 The Industrial Fisheries

The industrial fishery of Sierra Leone is dominated by foreign owned vessels, which employ only a limited number of Sierra Leoneans. The sector is capital intensive and constitutes the main source of revenue generation for the government as it is estimated to generate over US $ 8M annually (MFMR, 2018). The industrial vessel operates in the open deeper waters beyond the Inshore Exclusive Zones (IEZ) up to the Exclusive Economic Zones (EEZ) of 200 nautical miles. The fleets of the industrial fisheries include trawlers, shrimpers, purse seiners as well as carriers and motherships (MFMR, 2008). The production of the industrial fisheries fluctuates between 20,000 metric tonnes and 71,000 metric tonnes annually and is mainly exported with little or no value addition. The vessels must land a certain percentage of their catch in the country, except for the tuna vessels as there is no local market for tuna in Sierra Leone. Fish and fishery products from this sector are largely frozen on board (mainly whole, not gutted) and exported to Asia and the West African sub-region. Existing onshore facilities to process fish and to add value before export are limited and plans have been made to develop a fisheries harbour in Freetown which would reduce transhipment at sea and encourage the landing of fish caught by the industrial vessels. The industrial fisheries mostly exploit demersal stocks and shrimps. The dominant species are the Sparidae (*Dentex canariensis*) and crustaceans (shrimps) (Figure 3).



a. *Dentex canariensis* (Snapper) b. *Penaied notialis* (shrimp)

Figure 3: Snapper - *Dentex canariensis* (a) and shrimp - *Penaied notialis* (b)

2.1.3 The Aquaculture and Inland Fisheries

The aquaculture and inland fisheries are not fully developed and are practiced mostly in a few lakes and rivers, floodplains and swamps. Annual production for inland fisheries is currently estimated at 20,000 metric tonnes, of which about 5,000 metric tonnes come from lakes and 15,000 metric tonnes from riverine and flood plains (MFMR, 2018). There is room for increasing annual production to about 40,000 metric tonnes (MFMR, 2016). It operates at subsistence level and the fishing crafts used are mostly dug-out canoes and is done by poling in shallow waters and by paddling in deeper waters (FAO, 2008). The aquaculture fisheries are mainly a cultured fishery. The most common method is rearing fish in earthen ponds and the main species reared are the Nile tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*). National aquaculture production is limited and estimated at (250 Mt) 276 t/year and this is not accurately monitored (Showers, 2015).

2.1.4 The Government Fisheries Policy

The fisheries sector policy of Sierra Leone is geared towards fostering responsible fishing practices and sustainable development through good governance. It also seeks to improve national nutrition and food security through increased fish production and reduction of spoilage and wastage (post-harvest losses) as well as enhancing the socio-economic status of people in the fisheries sector, particularly women and children (MFMR, 2016). The policy accommodates the diverse and extensive natural resource governance issues. The policy framework has been strengthened to provide guidance for improved governance of tenure of fisheries in general; and to provide harmony, principles and guidance in governing small-scale fisheries in particular (MFMR, 2016).

2.2 Overview of the Quality Assurance of the Fishing Industries of Sierra Leone

Fish is a very perishable product and spoilage starts immediately after catching the fish. Once captured it is important to protect the fish from direct sunlight and chilling should be carried out quickly. About 80% of all fish consumed by Sierra Leonean is caught by the artisanal fishers (MFMR, 2008). Women as fish mongers are the major marketing agents of fish products throughout the country. This distribution is usually carried out in the urban cities and some provincial towns. These fish mongers can hardly get a regular supply of fresh and high-quality fish due mainly to poor handling and processing of the catch and lack of ice at strategic points. Fish handling and preservation is mostly carried out on board fishing vessels/boats or on land (Green et al., 2012).

Many artisanal fishermen in Sierra Leone are unable to handle their fish on board fishing boats after catch. Sometimes there is too much fish landed at one landing site especially at night when the boats return from their fishing trips. It is very common to see excess fish landed being buried in pits after spoilage. Though data collection is well established in the policy of the Ministry of Fisheries and Marine Resources, regular data collection is very expensive and quite often not achievable, example data on the value of wasted fish as a result of spoilage has never been collected. This is as a result of fish deterioration either at the storage point or in transit. Fish processing and preservation in Sierra Leone is traditionally by smoking, drying and freezing (Green et al., 2012). At the industrial level, fish and fishery products are blast frozen on board the fishing vessels and packaged normally in 20/25kg cartons for export. Smoking and drying methods are mostly practiced at the artisanal level for domestic and regional markets (Green et al., 2012).

The Fish Safety and quality assurance unit in the Ministry of Fisheries and Marine Resource (MFMR) has the sole responsibility of promoting responsible and sustainable fishing by identifying methods and procedures for assessing premises and equipment in line with national and international standards of operating procedures. Also reducing food processing related hazards and developing compliance verification for evaluation of conformity to general requirements in fish handling and processing establishments and fishing vessels. Ensuring effective control systems are essential to protect the health and safety of domestic consumers (MFMR, 2017).

Sierra Leone is presently not exporting fish and fishery products to the European Union (EU) due to EU Regulations relating to official controls and hygiene of food and feed (FVO, 2009). Based on the report, Sierra Leone needs to address official controls on fish and fishery products intended for the EU market including microbiological tests and chemical tests for histamine; organoleptic checks; contaminants including heavy metals and dioxins (EC 888, 2004); (EC 854, 2004) and (EC 853, 2004).

MFMR is working in collaboration with other ministries, departments and agencies (MDA's) in Sierra Leone such as the Competent Authority (CA) of the Ministry of Health and Sanitation (MOHS), the Sierra Leone Standards Bureau (SLSB) and the Ministry of Water Resources (MWR) to meet these requirements for export to the EU.

There are about eight land-based fish processing plants serving the industrial fishery. Presently in Sierra Leone most of the products are frozen and packaged onboard vessels for export. These products go mainly to countries such as South Korea, China, Liberia and Ghana. MFMR is working assiduously in addressing the lifting of the EU ban. Some of the major challenges faced in the industrial and artisanal fisheries sector include lack of dedicated fish harbour complexes for fishing vessels which require dry docking or bunkering (MFMR, 2018).

2.3 Commercially important fish caught in Sierra Leone

Sierra Leone has one of the richest marine fishing zones throughout West Africa and has a diverse and valuable array of fish stocks in marine and inland water environments. This is partly due to the migratory and multi-species nature of the resources (Jalloh, 2010). The fish stocks are mainly exploited through fishing and a limited number of aquaculture systems. The abundance of commercially important fish population is of key interest to not only fishermen but also to scientists alike to maintain the resource in a sustainable way (Mawundu, 2011).

The estimated current total annual production is about 222,000t with the largest single contribution coming from the marine artisanal fishing sub-sector (120,000t valued at USD 100 million per year at first sale). The catch from the marine industrial fishing sub-sector is estimated at 107,000t (valued at USD 81 million per year at first sale value) (MFMR, 2018).

Fish and other aquatic products are key for economic activity and as well as food security and nutrition. The fisheries sector contributes about 10% GDP to the national economy of Sierra Leone (Neiland et al., 2016). Fish is the most important source of animal protein in Sierra Leone. The main supply of fish comes from the marine fisheries, followed by the inland fisheries and a limited amount from the aquaculture sector. Roughly over 200 fish species have been identified and recorded in Sierra Leone's EEZ and these are mostly typical of the tropical fish communities. Over 100 of the marine fish species identified are commercially valuable and are exploited by industrial and artisanal fishers (MFMR, 2008). They are commonly found in Sierra Leone's domestic markets and exported internationally. Some of the relatively inexpensive species are the *Pseudotolithus typhus, Sardinella maderensis, Illisha africana and Ethmalosa fimbriata*. The high value species include the *Sepia spp*., *Octopus spp*., *Penaeus notialis*, *Cynoglossus spp*., *Solea spp*., *Pseudotolithus senegalensis*, *Dentex spp*., and *Epinephelus spp*. (Appendix 1). The catch composition of the major species varies from one fishing ground to another, seasonally and with depth. Marine fish species from both artisanal and the industrial fisheries are available throughout the year.

2.3.1 The Senegalese sole

The Senegalese sole (*Solea senegalensis*) (Figure 4) is a demersal species found naturally in Atlantic and Mediterranean waters. It is a potentially important species for marine aquaculture owing to its high market value and consumer demand as compared to *Cynoglossus senegalensis* (Tongue sole) a marine species found at a depth range of 10 – 110 m on the sand and mud bottoms of coastal waters. The market value of *Cynoglossus senegalensis* (Appendix 2) is very questionable because it is based on ex-vessel price for the species (Froese & Pauly, 2018).

Senegalese sole is better adapted to the warmer waters of temperate climates, therefore more suitable for production. Senegalese soles are easily distinguished from most common sole fish by the black colouration of its interradial membrane on the pectoral fin on the eyed-side. *S. senegalensis* is a flatfish with an oval and asymmetric body (eyes on the right side). The blind-side of the head is covered with numerous small hair-like fringes and the upper eye is separated from the dorsal profile of the head by a distance distinctly greater than the diameter of the eye. The anterior nostril of blind-side is surrounded by a small ridge but not enlarged. The primary habitat is sandy or muddy bottoms ranging from brackish lagoons and shallow waters to coastal areas up to a depth of 100m (FAO, 2018).

Senegalese sole is gonochoric, having separate males and females and the female attains sexual maturity at around 2 years age and a total length of around 30-40 cm but can reach up to 70 cm. Senegalese sole weighs approximately 350-400g. Spawning occurs in spring (March-June) and is obtained at temperatures between 16^0C and 22^0C. The sex ratio is two males to one female (FAO, 2018).

Figure 4: The Senegalese sole (*Solea senegalensis*)

The black colour of the membrane between the rays on the pectoral fin on its eyed-side differentiates it from the common sole (FAO, 2018).

3 LITERATURE REVIEW

3.1 Shelf Life and spoilage of fish

Shelf life of food is defined as the maximum length of time food is fit for human consumption. Food is perishable and numerous changes take place during processing and storage. The shelf life of fish count from once the fish is taken from the water until it is no longer fit for human consumption. Fish is highly perishable, and freshness is a major contributor to the quality of fish products. Handling on board and on land by technological processing influence the freshness of seafood, as well as other handling practices (Martinsdottir, 2002).

Temperature is one of the most important factors determining the shelf life of fish. Low temperature maintenance inhibits or reduces bacterial growth and enzyme breakdown. Fresh fish exposed to ambient temperature even for a short period of time will deteriorate faster than if kept constantly at a cold temperature. Keeping freshly caught fish at temperatures close to 0 °C from the fishing vessel to the processing plant is usually challenging (Chatzikyriakidou & Katsanidis, 2011).

Fish spoilage begins as soon the fish dies. A series of deteriorative changes result in spoilage. Autolytic, microbiological and chemical phenomenon are responsible for spoilage in fish and fishery products (Huss, 1995). These changes can be delayed or accelerated by physical conditions like temperature, pollution, physical damage and contamination by bacterial flora. Spoilage is usually accompanied by change in physical characteristics such as colour, odour, texture, colour of eyes, colour of gills and softness of the muscle. These are some of the characteristics observed in spoiled fish. The activity of these organisms can be controlled, reduced or even retarded by proper handling and immediate lowering of the temperature. The antibacterial compounds found in the slime of the thick skin of flatfish may also contribute to the keepability of flatfish. In general, the slower rate of spoilage of some fish species has been attributed to slower bacterial growth. Different spoilage rates seem to be related at least partly to the rate in increase of bacteria on them. Fish taken from warm waters keep better than fish from temperate waters. Studies have shown that the longer shelf lives are found in fresh water fish species compared to marine species. Although there are very wide variations, studies show that tropical fish species often have prolonged shelf lives when stored in ice. An absence in development of trimethylamine (TMA) and total volatile nitrogen (TVN) during storage shows that spoilage of tropical fish is not caused by bacteria (Huss, 1995).

The chilling of the fish immediately after catch and holding the fish at 0 ^{0}C by proper icing will reduce the spoilage (Huss, 1995). Various factors such as species type and different storage conditions influence the spoilage pattern of fish product resulting in loss of freshness and the spoilage of the fish. Freshness period (FP) of fish products can be defined as the time from processing/packaging until the product losses its freshness characteristics. The remaining shelf life (RSL) will be the time the fish product reaches sensory rejection. Therefore, the sum of FP and RSL will give the sensory shelf-life of the products (Lauzon et al., 2010).

3.1.1 Autolytic spoilage

The initial quality loss in fish freshness is due to autolytic changes. This is not related to microbiological activity. It contributes very little to spoilage of chilled fish and fishery products. Autolytic spoilage basically is the degradation of trimethylamine (TMA) by autolytic enzymes to dimethylamine (DMA) and formaldehyde (FA). The FA formed caused an undesirably dry and hard fish texture (Huss, 1995).

3.1.2 Microbiological spoilage

On live and newly caught fish, microorganisms exist on the skin/slime, gills and the gut. The bacterial flora on newly-caught fish depends on the environment in which it is caught rather than on the fish species. These organisms vary depending on the environment the fish is caught and the proportion on the surface and gills/gut of the fish. The bacteria loads can range from 10^2-10^7 cfu/cm^2 on the skin surface and 10^3 to 10^9 cfu/g both in the gills and intestines. Very high numbers, i.e., 10^7 cfu/cm^2 are found on fish from polluted warm waters. The psychrotrophic Gram-negative of the genera *Pseudomonas, Shewanella, Vibrio* etc. are dominant bacteria found on temperate water fish. Gram-positive (*Bacillus, Micrococcus*) dominate on fish from tropical waters. Fish caught in warm waters carries a slightly higher count of bacteria flora as compared to fish caught in very cold, clean waters. Studies have shown that that *Escherichia coli* and *Salmonella* can survive for very long periods in tropical waters and once introduced may almost become indigenous to the environment (Sveinsdottir, Hyldig, Martinsdottir, Jørgensen, & Kristbergsson, 2003).

Autolytic changes cause the initial loss of fish quality whether chilled or not chilled while spoilage is mainly due to bacteria breakdown. Many fish preserved at temperatures above 0^0 C spoil as a result of microbial activities. During storage a characteristic flora develops. Only a portion of this flora contributes to spoilage (Huss, 1994). For fish living in temperate waters, spoilage is characterised sensorially by the development of offensive fishy, rotten, H_2S off-odours and off-flavours. This is different when compared to tropical and fresh water fish when sensory characterised like fruity, sulphydryl off-odours and off-flavours (Gram & Huss, 1996).

(Gram & Huss, 1996) reports that microbial spoilage of food can take diverse forms which are all the consequences of microbial growth. They are manifested as changes in the sensorial characteristics (Table 1).

Table 1: Microbiological Spoilage of Food (Gram & Huss, 1996).

Microbiological Activity	**Sensory Manifestation**
Breakdown of food components	Production of off-odours and flavours
Production of extracellular polysaccharide material	Slime formation
Growth per se of moulds, bacteria, yeasts	Large visible pigmented or non-pigmented colonies
Co_2 ---------form carbohydrate or amino acids	Production of gas
Production of diffusible pigments	Discoloration

3.1.3 Chemical spoilage

One important aspect of fish quality is the chemical composition. The chemical composition of the fish plays an important role in the quality of the fish. Fish have a higher degree of unsaturated lipids than other food commodities and are known to be susceptible to oxidative rancidity during storage (Huss, 1995).

Fish lipids are subjected to two main processes: auto-oxidation and lipolysis. Hydroperoxides are formed through a process involving atmospheric oxygen and fish unsaturated lipids which are associated with a tasteless flavour accompanied by brown and yellow discolouration of the fish tissues. The importance of these reactions mainly depends on the fish species and the storage temperature. Further breakdown of the hydroperoxides gives rise to the formation of strong rancid flavours (ketones and aldehydes). Several factors such as organic and inorganic (copper or iron), heat and light can initiate and accelerate oxidation (Huss, 1994).

3.2 Effect of temperature on the shelf life of fish

Temperature greatly influences both enzymatic and microbiological activity. It is the most significant factor influencing spoilage. The most important factors determining the quality of fish and fish products are time and temperature tolerance. Temperature changes have a greater impact on microbiological growth than on enzymatic activity. The rapid reproduction of microorganisms requires appropriate high temperatures, while at lower temperatures close to 0^0 C the reproduction of these microorganisms will reduce, hence extending the shelf life of fish products (Amos, 2007).

At 0-25^0 C, microbiological activities are very important in the shelf life and spoilage of fresh fish stored at chill conditions in melting ice and ambient temperature approaching 0^0 C and between 10-30^0 C for warm/tropical countries. Many bacteria are unable to grow at temperatures below 10^0 C (Huss, 1995).

Improper chilling or temperature abuse will shorten the freshness period of the fish products whereas superchilling (0^0 C to -4^0 C) will extend it. Super chilling has proven to be more effective slowing down bacteria growth and extending the shelf life of chilled fish and fish products. Temperature abuse can be harmful on uncooled fish products or in those cooled but produced under poor hygienic conditions. This often leads to high microbial contamination. Delay in icing fish immediately after capture will lead to microbial proliferation, chemical and biochemical degradation of fish, hence reducing the shelf-life of fish on ice. Studies have shown that fillets stored at 2^0 C and combined contact cooling (CBC) fillets stored at -0.9^0 C showed a freshness period of 7.5 days and a shelf life of 10 days respectively with both resulting in a similar shelf life of 10 days. By lowering the temperature of the raw material to 0.4^0 C and the CBC fillets to -0.3^0 C the freshness period for both fillets was increased by 12.5 days and a shelf life of 13 days. The results show that lowering the average temperature of the fillets thus increases the freshness period and the shelf life (Lauzon et al., 2010).

In the case of temperature abuse chilled fresh filets stored a temperature of 1.9 ± 2.3^0 C in 3kg expanded polystyrene (EPS) boxes and processed from 1-day old cod for 16 hrs at room temperature, the freshness period reduces by 2.5 days and the shelf life by 3 days compared to chilled CBC fillets where the temperature abuse led to a decrease in the freshness period from 10 days (EPS boxes) to 8 days (Lauzon et al., 2010).

The microflora responsible for spoilage of fresh fish changes with changes in storage temperature. Temperature and handling practices are the most important factors affecting post-harvest quality of the products. An indisputable fact is that the higher the temperature, the faster the fish spoil due to increase in bacterial action. Fresh fish are highly perishable, it is mandatory for the fish industry to market the highest quality product as quickly as possible. This strategy will yield the greatest economic return to the industry. Therefore, it is to the industry's advantage to make every effort to extend the shelf life of all fish and fish products. The overall reason for icing fish is to extend fresh fish shelf life as compared to storing un-iced fish at ambient temperatures above 0° C. However, extending shelf life is basically to produce safe fresh fish of acceptable quality (Huss, 1995).

3.3 Methods used to evaluate fish freshness

In assessing the quality of fish and fishery products, freshness is the most important attribute. For all kinds of fish and fishery products, freshness makes a major contribution to the quality of fish and fishery products (Ólafsdóttir et al., 1997). Many methods have been tested to evaluate the quality of fresh fish or measure parameters that change, form or disappear during fish deterioration. Sensory methods are still the most satisfactory way of assessing fish freshness and its deterioration. Sensory methods must be performed scientifically and under careful conditions, reducing personal bias and the effects of the test environment. Methods to verify freshness are needed at different transaction points in the fishery chain from catch to consumer, at landing site and auction. Its needed at all levels of trade from auctioning through wholesale to retail (Martinsdottir, 2002).

3.3.1 Sensory Evaluation methods

Sensory changes in food are perceived with the human senses such as sight, smell, taste, touch and hearing. Sensory evaluation is defined as a method used to measure, evoke, analyse and interpret reactions to the characteristics of food as they are perceived through the senses of sight, smell, taste, touch and hearing. Food products under controlled conditions can be analysed statistically when evaluated by applying sensory evaluation. Sensory evaluation is one of the most important methods for assessing freshness and quality in the fishing sector and in fish inspection services. It takes place on all levels from processing plants, marketing from auction sites, wholesale to retail level (Sveinsdottir et al., 2003).

Sensory evaluation methods are the highly realistic way of assessing freshness of fish and fishery products. This is because they can be applied to all types of fish species. The method is easy, non-destructive and quick to use and does not require sophisticated laboratory facilities. Sensory evaluation relies on trained panellists to perform the sensory analysis. To get good results with sensory evaluation, the panellist must be trained and have a clear and descriptive guideline on the raw and cooked products. Inspectors performing such duties need training under supervision of experienced leaders (Olafsdottir et al., 1997). There are several methods used to determine freshness of seafood products and regulations dictated by countries. The European Union (EC 2406, 1996) have laid down marketing standards for fish and fishery products entering the European Union. It has three (3) grading scheme for freshness rating corresponding to various stages of spoilage. The rating grades are Extra E, A and B (given according to sensory evaluation of gills, smell, skin, eyes, flesh and other characteristics). Extra (E) is the highest possible quality while the product is discarded or rejected when the grade is below B. There are schemes (The EU-scheme) for different groups of fish and size categories. This does not consider difference between species but include general parameters for each group of fish (cephalopods, crustaceans, whitefish, selachii and bluefish) (EC 2406, 1996). The scheme gives limited information about the condition of the fish (Kilcast, 2010).

3.3.2 Quality Index Method (QIM)

The Quality Index Method (QIM) is a relatively new and improved seafood freshness and quality grading system. It is rapid, objective and has several unique characteristics. The QIM must be adapted to each species (Martinsdottir. E, 2010). The method mostly used and recommended in Europe today for quality assessment of raw fish in the industry and inspection services is the EU scheme. This scheme is very general, not species specific and provides limited information about the shelf life. Evaluating the primary methods to measure freshness of fish at different temperature levels hence predicting the shelf life is key in fish safety and quality management. Fish and fishery products pricing depends on its freshness and quality (Martinsdottir, Sveinsdottir, Luten, Schelvis-Smit, & Hyldig, 2001).

The Quality Index Method (QIM) is a seafood freshness quality control system and easy to use. It is a non-destructive, simple, and cheap method in assessing freshness changes of fresh fish and no equipment is needed more than human senses. It considers specific aspects of each species, or products, assessing the quality and freshness of the fish by sensory analysis. With QIM, processors can estimate the shelf life of fish products which may result in more production control. QIM is easy to teach and easy to understand for inexperienced people to evaluate the fish. Quality Index increased linearly with the storage time on ice making it possible to estimate the remaining shelf life of the fish product (Sveinsdottir et al., 2003). The QIM appears to be an easy, rapid and efficient tool to assess the storage history and estimate the remaining shelf life of the fish taking into consideration the high demand for information about quality and freshness of fish by consumers (Bernardi, Mársico, & Freitas, 2013) . QIM is based on characteristic changes that occur in raw fish and a score system from 0 to 3 demerit (index) points. Each quality parameter is divided with a description of each. The scores of all the characteristics are summarised to give an overall sensory score, called the quality index (QI). A Ql of zero (0) is given for very fresh fish and the Ql score increases as the fish deteriorates. It is possible to predict the remaining shelf life when the fish is stored in ice with the QI. One of the objectives for the development of the scheme is to develop a linear correlation between the sensory quality (QI) and the storage time in ice which makes it possible to predict the remaining shelf life in ice. A QIM score of zero (0) gives very fresh fish and a higher score results as the fish deteriorates (Sveinsdottir et al., 2003).

QIM has been developed for several fish species. A reference manual by the EU for the fisheries sector has been published including QIM scheme for seafood species and sole (Martinsdottir et al., 2001) and is available in 11 languages. A further scheme has been developed for Senegalese sole (Gonçalves, Antas, & Nunes, 2007).

3.3.3 Torry Scale methods

The Torry freshness score is a systematic scoring system which is based on an objective sensory method to assess the state of the fish or the freshness of the fish. The grading scheme describes different sensory attributes such as odour, texture and appearance. It is common to cook the fillets and evaluate their odour and flavour. The Torry scale method is widely used for evaluating freshness of cooked fish and in the fish industries by both buyers and sellers of fish products. It is a descriptive 10-point scale that has been developed for lean, medium-fat and fat fish species. The scale scores are given from 10 (very fresh in taste and odour) to 3 (very spoiled) and below 3 is considered unnecessary, as the fish is then no longer fit for human consumption. Cooked samples of fish fillets can be used to determine the maximum storage time of the fish. An average score of 5.5 has been used as the limit when the fish is unfit for human consumption. At this stage, there is evidence of spoilage characteristics such as sour taste and hints of off-flavours (Martinsdottir et al., 2001).

3.3.4 Generic Descriptive Analysis (GDA) Method

Generic Descriptive Analysis (GDA) is a method used by a sensory panel to evaluate cooked fish. It is a total sensory description that considers all sensations that are perceive –odour, appearance and texture. A basic strength of GDA method is the ability to independently verify (after each test) that persons perceive differences amongst products on attributes in a reliable manner. The panellists are trained according to International Standards (ISO, 1994) for the GDA assessment. GDA is directly measured with analysis of variance from each panellist for each attribute and there is a need to monitor performance of each panellists in each test. This reflects the awareness of sensory limitation by man (Lawless & Heymann, 2010).

3.3.5 Microbiological Methods

Microbiological examination in fish aims at evaluating the hygienic quality of the fish which includes temperature abuse and the presence of pathogens in the fish. Thus, microbes play an important role in the degradation of fish products (Huss, 1995). A better knowledge of the microbiological conditions throughout the fish production chain may help to optimise product quality and resource utilisation (Svanevik, Roiha, Levsen, & Lunestad, 2015). Contamination by microorganisms may occur through contact with surfaces of handling equipment, personnel, water and storage during landing and processing of fish and fishery products. Their activity is the main factor limiting shelf life of raw fish. Newly caught fish contains a diverse microflora and are found on the outer surface of the gills, skin and the intestines. An estimation of the total viable counts (TVC) is usually used as an acceptability in standards, guidelines and specifications. A total of $10^2 – 10^6$ cfu/g TVC are usually on whole fish and fillets stated as a normal range. A TVC of fish products with $10^7 – 10^8$ cfu/g is at the point of sensory rejection (Olafsdottir et al., 1997). TVC can give controversial estimation of the end of shelf life. Different limits have been set based on the product type. The number of specific spoilage bacteria (SSO) in fish products will give information on the remaining shelf life and this can be estimated from such numbers. Improving the hygiene conditions at some critical points at the early stage of the production will improve the quality of the products. Thus, proper temperature control is essential to minimise the bacterial growth prior to freezing. Hence, bacterial established at the early stage of processing may be retained and controlling bacterial contamination is very important from harvesting to processing so the quality and safety of the end products will not be adversely affected (Olafsdottir et al., 1997).

3.3.6 Chemical Methods

Besides sensory, physical and microbiological methods, chemical analyses have been used to assess the freshness quality of fish. During storage there are changes in seafoods due to autolytic enzymes, microbial activity or chemical reactions that can be useful indices of quality or spoilage that can be measured with chemical methods. The chemical composition of fish varies greatly from species to species and one individual to another depending on the age, sex, season and environment (Huss, 1995).

In evaluating fish quality, a total number of spoilage indicators have been used such as trimethylamine (TMA), total volatile basic nitrogen (TVB-N) and the formation of biogenic amine. The total levels of TVB-N and each of these compounds are useful indices of spoilage in seafoods. The European Commission (EC 149, 1995) specified TVB-N to be used if sensory evaluation indicates doubt about freshness of different fish species. Critical limits of 25, 30 and 35 mg-TVB-N/100g were established for different groups of fish species (Dalgaard, 2000).

In the case of fish freshness, the nucleotide degradation product ratio such as K, Ki values and hypoxanthine (H_x) have been used in determining freshness. The K-values increase linearly during the first days of storage often giving an excellent index of freshness. The K-value is not considered reliable and cannot be used in general as an index of spoilage for all fish species because many fish species reached their maximum levels long before sensory rejection (Dalgaard, 2000).

Lipid oxidation is a major cause of deterioration and spoilage for fish with high oil/fat content stored in their flesh. Lipid oxidation can occur enzymatically or non-enzymatically. This is done by the breakdown of fats and other lipids by hydrolysis to release fatty acids. They form free fatty acids which are responsible for common off-flavour (rancidity) and the reduction of the oil quality in fish (Martinsdottir et al., 2001).

4 MATERIALS AND METHODS

4.1 Senegalese sole

Fresh whole Senegalese sole from Grindavik/Stolt Seafood arrived at Matís Reykjavik on the 6^{th}, 11^{th}, 13^{th}, 18^{th}, 20^{th}, 21^{st} and 27^{th} December 2018 and 9^{th} and 16^{th} January 2019. The fish was in polystyrene boxes covered with thin plastic film with flake ice (0^0C). At the laboratory the fish were stored at room temperature of 2.5±0.5°C and re-iced as needed during the experiments until sensory rejection.

Four different batches of fresh Senegalese sole used for this study were obtained from Grandavik/Stolt Seafood. A total of nine (9) commercial size (350±50g) were used in the three storage trials for the first practical session and twelve (12) fishes used in the four storage trials in the second practical session (Table 2). The fish were slaughtered at Grandavik/Stolt and packed in polystyrene boxes covered by thin plastic film with flake ice (0^0C) and transported to Matís – Icelandic Food and Biotech R&D the same day of harvesting. The polystyrene boxes were provided with holes for drainage. The fish were held un-gutted in polystyrene boxes stored under refrigerated conditions at 2.5±0.5°C, and the ice was replenished as required.

Two groups of fresh whole Senegalese sole were prepared, and both groups stored at 0^0C for one day. On the second day, one group was transferred from the cooler and abused at 20^0C for 16hrs (at room temperature). The samples were then moved back to the cooler and stored at 0^0C. The samples were analysed regularly until sensory rejection.

Table 2: Sampling plans of farmed fresh whole Senegalese sole (QIM/Torry) on pre-observation, panellists training and shelf life study.

Type of session	Date	Number of fishes evaluated	Storage days
Pre-observation	6^{th} December 2018	3,3	8,13
	11^{th} December 2018	3,3	3,6
	13^{th} December 2018	3,3	1,3
	18^{th} December 2018	3	1
Panellists training	27^{th} December 2018	2	21
	3^{rd} January 2019	2	15
	9^{th} January 2019	2	8
	13^{th} January 2019	2	2
Shelf Life study	18^{th} January 2019	10	2
	23^{rd} January 2019	10	7
	28^{th} January 2019	10	12
	1^{st} February 2019	10	16

4.2 Experimental design

The study was undertaken in two phases. The first phase was the development of the QIM scheme, including a pre-observation of the fish with storage time and training of panelists, and a finalisation of the sensory scheme for Senegalese sole and the second phase was the shelf-life experiment Figure 5. During the pre-observation, 3 fishes per storage day were evaluated. The trial was done to obtain some specific knowledge about the fish species. A detailed description during the complete ice storage until the end of an expected shelf life was done. For the training two (2) fishes per storage day were evaluated.

In the shelf life study, 10 fishes were evaluated and chosen randomly for sensory, chemical and microbiological analysis. The modified QIM scheme was used to evaluate the whole fish in four sessions. Eight to eleven trained panellists carried out the evaluation. Each panellist evaluated ten whole fresh fish per storage day (2,7,12,16) coded with three-digits numbers without knowledge about the storage time.

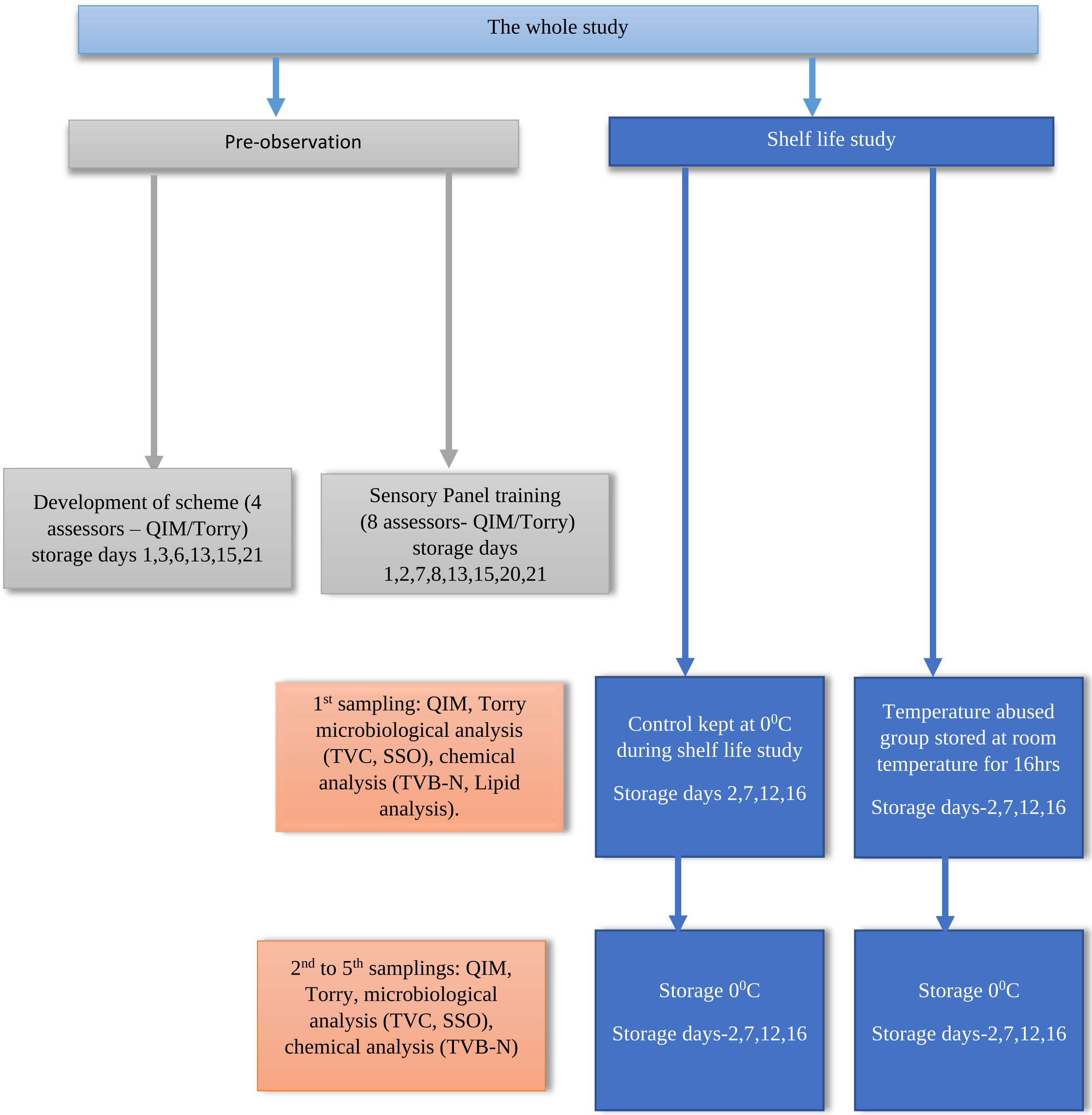


Figure 5: Development of QIM scheme and shelf life study for farmed fresh Senegalese sole using Quality Index Method (QIM) scheme developed.

4.3 Temperature Loggers

Temperature mappings during the shelf life study were performed with iButton® DS1922L temperature loggers to monitor the temperature of the whole fresh Senegalese sole and the ambient storage temperature. These loggers have an accuracy of ± 0.5°C, a resolution of 0.0625 °C and an operating range of -40 to 85 °C. The diameter is 17 mm and the thickness of 5 mm. Four set of temperature loggers were located inside (control and abused) and another four set outside (control and abused) the polystyrene boxes. Temperature loggers were used to monitor the temperature of the whole fish and the ambient temperature of the environment from the 17th January 2019 to 1st February 2019.

4.4 Development of QIM scheme for fresh Senegalese sole

4.4.1 Pre – Observation

During the pre-observation, a preliminary QIM scheme for the sensory evaluation of fresh whole Senegalese sole was designed. The QIM scheme developed was based on previous work already published by (Gonçalves et al., 2007) and the Eurofish QIM scheme (Martinsdottir et al., 2001) found on the site of the international project QIM-Eurofish for fresh whole sole (Appendix 3).

A total of 21 whole fresh Senegalese sole fish were observed in the sensory laboratory using QIM scheme published by (Gonçalves et al., 2007) and the QIM for sole (Martinsdottir et al., 2001) as support material (Appendix 3). Two practical sessions were done for the pre-observation. The fish were placed on a white clean table under white fluorescent light and labelled with storage days (Figure *6*). The sampling days were 1, 3, 8, 6 and 13. Changes (in eyes, skin, gills, and texture) occurring in the pre-observation was assessed and registered by four persons and the parameters evaluated based on freshness to spoilage of the fish were determined. During this time, the QIM scheme by (Gonçalves et al., 2007) was slightly modified and used to train panelists.

Parallel to the pre-observation of the whole fresh Senegalese sole, sensory evaluation of the cooked Senegalese sole was done using Torry scale for medium fat fish and fatty fish published by (Martinsdottir et al., 2001). Five fish samples in each of the two sessions were filleted and four 30-40g samples collected and placed in aluminum boxes and cooked in a pre-warmed electric oven Convostar (Convotherm-German) by steam at 150^0C for 6 minutes and then served to the panelists. The Torry scale for fatty fish was used at the end of the pre-observation session since it was better fitted with the sensory characteristics of the fish (Table 3).

Figure 6: Preparation for evaluation of whole fresh fish (QIM)

4.4.2 Training of Panelists

The scheme developed was used in order to train the panel. The sensory panellists were previously selected and trained in line with international guidelines and standards (ISO, 1994) and (ISO, 1993). Eight (8) sensory panellists were trained on the new QIM and at the same time evaluating the scheme during the two sessions on QIM, Torry method, and GDA for texture. The panel leader explained to the panellists how to use the scheme and the evaluation methods.

For the QIM training, three fish samples per storage were used and during the training, fish stored 1, 2, 7, 8, 13, 15, 20, and 21 days in ice was used. Whole farmed fresh Senegalese sole of different storage time were placed on white clean table under white fluorescent light and the storage day given with a note next to each fish during the training session as shown in (Figure 7). A modified Quality Index Method (QIM) scheme was used to evaluate freshness changes of the whole farmed fresh Senegalese sole with storage time as described in the QIM reference manual of farmed Senegalese sole (Goncalves, Antas, & Nunes, 2007). The panellists were trained on the descriptors and quality parameters of Senegalese sole. Suggestions to the scheme developed were made by the panellists and were incorporated. The total demerit points first established by (Gonçalves et al., 2007) was 28. During the development of the scheme, some parameters were found not useful such as the flesh. The modified scheme had a total score of 26 demerit points instead of 28 demerit points.

Figure 7: Senegalese sole coded with storage day (Day 7)

4.5 Shelf life study of fresh Senegalese sole

4.5.1 Sensory evaluation of whole fresh Senegalese sole with QIM

The QIM method by which sensory evaluation is performed in a systematic way by objectively assessing the quality attributes of the freshness of fish species. Every sampling day, eight trained panellists evaluated the fish with the modified QIM scheme. Sensory evaluation of whole farmed fresh Senegalese sole was done in the sensory laboratory of Matís. The samples were placed on a clean white table 15 minutes before the evaluation at room temperature under white fluorescent light. The samples were coded randomly with three-digit numbers. Five samples from each storage temperature (control and T-abused) were evaluated for four sessions. Eight trained panellists assessed changes in gills, colour, texture, skin, mucus and odour of fish. The panelists were experienced in sensory analysis and familiar with QIM method. All panellists evaluated all the Senegalese sole by moving from one fish to the other and registered their evaluation for each quality parameter in a QIM scheme.

4.5.2 Sensory evaluation of cooked Senegalese sole

Seven trained panelists from Matís ohf. were trained during two sessions to evaluate cooked Senegalese sole fillets according to Torry for fatty fish and selected texture attributes (GDA) Appendix 6. 30-40g of filleted samples collected from two different storage temperature (control and T-abused) were placed in aluminum boxes (Figure *8*), coded with three-digit random numbers and cooked in a pre-warmed electric oven Convostar (Convotherm, Eglfing, Germany) by steam at 150^{0}C for six (6) minutes with air circulation and steam. The boxes were closed with plastic lids and then served to the panellists. The sensory evaluation was carried out in a special room equipped with individual booths and conducted according to international standards (ISO, 1998). The panel observed and evaluated the cooked samples for difference in odour, texture and flavour without knowledge about the storage time. Each sample was evaluated randomly in duplicate and average scores of the judges were calculated for each sample assessed. The 10 score on the Torry scale indicated very fresh fish, but 3 was spoiled, with sour, spoiled fruit, rancid and ammonia. The average score of 5.5 may be used as the limit of acceptability (Martinsdottir et al., 2001).

Generic Descriptive Analysis (GDA) on selected texture attributes was used parallel to the Torry score method. The panellists have experience in recognition of sensory characteristics of the samples and to describe the intensity of each attribute using an unstructured scale from 0 to 100. The panellists evaluated the cooked samples using 5 texture attributes on a 15 cm line scale from (Appendix 6). The assessment was carried out in a sensory laboratory equipped with separate booths. Panellists enter the data into the computer, that are collected individually from the panellist as they are completed.

Figure 8: Sample preparation of cooked fillets

Table 3: Torry scale for fatty fish (QIM- Eurofish)

Odour	**Flavour**	**score**
Butter, margarine	Meaty, shellfish flavour, slightly bitter, slightly garlic flavour	10
Fatty odour, peppery	Fat reminds of herring, metallic but meaty	9
Fatty odour, “backed” odour, peppery	Spiced meat, garlic, peppery	8
Caramel, boiled potatoes, musty	Neutral, slightly sweet flavour	7
Metallic, slightly sour	Insipid (towards ‘off’-flavour), slightly rancid sour or bitter	6
Milk jug odours, reminiscent boiled clothes	Slightly sourness, traces of ‘off’ – flavours, rancid	5
Sour beer, TMA-ammonia, spoiled cheese	Bitter, sour, traces of TMA, rancid, ‘off’ – flavour	4
Ammonia, very sour, drain-odour	Strong brightness, sour, spoiled fruit, rancid	3

4.5.3 Chemical Analysis

4.5.3.1 TVB-N

Total volatile nitrogen (TVB-N) was measured in the Senegalese sole during the shelf life study. Steam distillatory method was performed using a Kjeldahl type distillator (Struer TVN) to analyze TVB-N for whole fresh Senegalese sole. 50g of fish sample was minced and mixed with 100 ml of 75% aqueous trichloroacetic acid solution homogenized and blended for 1 minute and filtered using a Whatman filter paper. 25ml of the filtrate was transferred into a distillation flask and 6ml of 10% of NaOH was added to the filtrate. The distillate collected into Erlenmeyer flask and 10ml of 4% boric acid and 0.04ml of methyl red and bromocresol green indicator and placed under the condenser for the titration of ammonia. Distillation was done for 4 minutes using Kjeldahl type distillator (Struer TVN). The boric acid solution turns green with aqueous ca. 0.025 N sulphuric acid solution using a 0.5 ml graduated burette. Complete neutralisation was obtained when the colour gradually turned from grey to pink on the addition of a further drop of sulphuric acid. Result calculated as the TVB-N (mgN/100g) (Malle & Poumeyrol, 1989).

Calculation (mgN/100g): $$\frac{14\text{mg/mol}*a*b*300}{25\text{ml}}$$

a: ml of sulphuric acid

b: Normality of sulphuric acid

4.5.3.2 Lipid Analysis

The fat content of Senegalese sole was determined by the AOCS Official method Ba–3–38 with modification according to Application note Tecator no AN 301 according to the Matís Icelandic Fisheries Laboratory. A 5g sample of minced Senegalese sole was wrapped in a filter paper and 80ml of petroleum ether was added to the sample and placed in a Butt extraction tube. The sample was heated in an electric hot plate at a temperature range of 40–60^{0}C whiles the petroleum ether from the extraction evaporated. The extraction was collected in a beaker and weighed, and the percentage of the fat content calculated (AOCS Ba-3-38, 1997).

4.5.4 Microbiological Analysis

On each sampling day, four fish from each storage temperature (control & T-abused) were collected and taken to the microbiological laboratory of Matís. The fish samples were minced and weighed, 25g of minced fish were placed in a stomacher bag and mixed with 180g of Butterfield's Buffer solution and blended in the stomacher for one minute. Serial dilution into10-folds are made. Aliquot ml is pipetted into petri dish and melted agar are poured into the petri plates, mixed and allowed to set. The plates are incubated at 17^{0}C for 5 days. The total viable counts TVC (both white and black) and selective counts of H_2S-producing bacteria were done on Iron agar (IA) by the pour plate technique with an overlay (NMKL Method No 136, 2010).

4.6 Data Analysis

Microsoft Excel 2016 (Office 365) was used to calculate the means for TVB-N, QIM, and TVC and to generate graphs against the storage time. It was used to fit linear regression and correlation equation on QIM.

The results of GDA were treated with analysis of variance (ANOVA) to analyse if some difference existed between the samples. In the ANOVA when $p < 0.05$ there are significant differences between samples.

5 RESULTS

5.1 Development of QIM for Senegalese sole

5.1.1 Preliminary QIM Scheme

During the pre-observation, parameters that describe changes in skin, eyes and gills were listed in a preliminary scheme for the whole fish and the first modifications to the original QIM scheme was done (Table 4). A scheme was proposed based on the pre-observation of the Senegalese sole. Several descriptions were in line with the Eurofish QIM scheme (Martinsdottir et al., 2001) and (Gonçalves et al., 2007). Each parameter was assigned with descriptive scores (0-3). A total sum of 28 points was recorded (Table 4). Comparing scheme by (Gonçalves et al., 2007) and that developed from the pre-observation, (Gonçalves et al., 2007) has a higher number of parameters (11 instead of 10) and a higher total demerit points of 28 instead of 26 (Appendix 3). The modified scheme was used in the training of panellists and additional changes were made as was necessary to adjust the scheme.

5.1.2 Finalization of QIM scheme

During the training sessions, the panelists started the sensory evaluation using the preliminary QIM scheme developed. Suggestions to the preliminary QIM scheme were provided by the panel and these were considered. The descriptions of skin appearance, eyes and gills were modified to better define the changes. The average QI was calculated for the different storage days (1,7,13 and 20) of the fish. There was a high correlation between the average QI and days in ice (R^2 =0.9869) Figure 9. The quality parameters for flesh were removed and spot on the ocular side made to stand alone as a whole parameter. It was observed that the mucus was more visible in the beginning of storage and becomes less with storage time Figure 11. The bluish/whitish spots on the ocular side were the earliest and most evident changes found in the skin appearance. The spots are visible when the fish is fresh but fades out slowly with storage time Figure 12*Figure 12*. The eyes were flat, socket convex and pupil is black, clear and with a golden rim around the pupil at the beginning of storage. At the end of storage, the eye became swollen, socket sunken and pupil milky with a faded golden rim and pinkish/yellowish color of the iris (Figure 13). The skin lost its brownish pigmentation and became pale and purple discolored at the edge of the fins with storage time (Figure 14).

After the last training session, the scheme was modified and total sum of 26 points was recorded (Table 5).

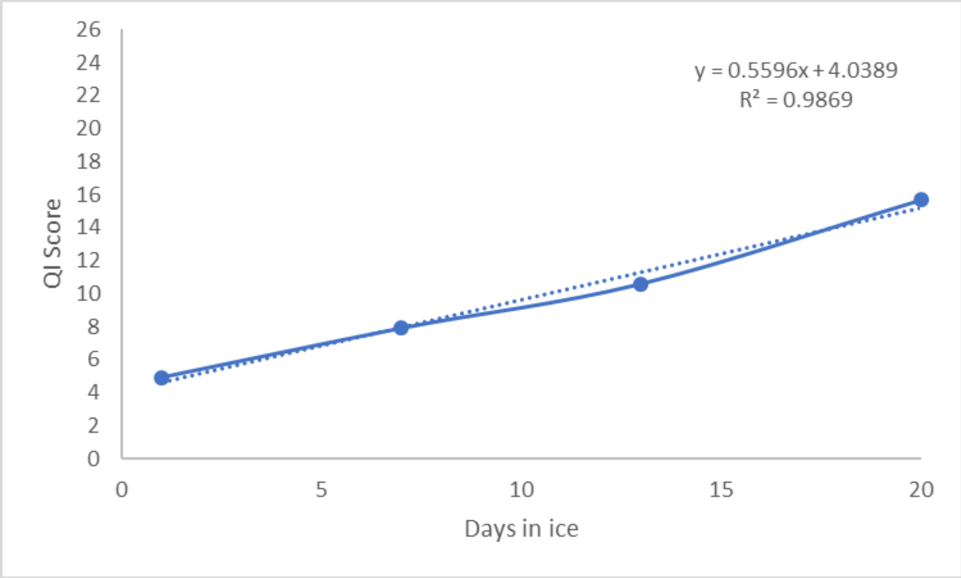


Figure 9: Average scores versus storage days in ice for Senegalese sole during the training

The average QI scores for spots on ocular side increased on day 7 by 1.6 but decreased on day 13 by 0.5. The scores increased until the end of the storage time. At the beginning of storage, the gills' colour scored an average of 0.7 (dark purple/dark red) and by the end of the training, the average score was 1.5, described as yellowish/brownish discoloration. The eye form (clarity and colour) were the quality attributes that presented most difficulties for panellists during the evaluation. Overall, there were some variation in the QI scores by all the panellists. Some panellists gave higher scores during the early storage while some scored lower at the same storage time as this was evident by the averages scored by the panellist four with lower scores (Figure 10). At the end of storage, the panellists were in more agreement as is indicated from the QI scores (Appendix 4).

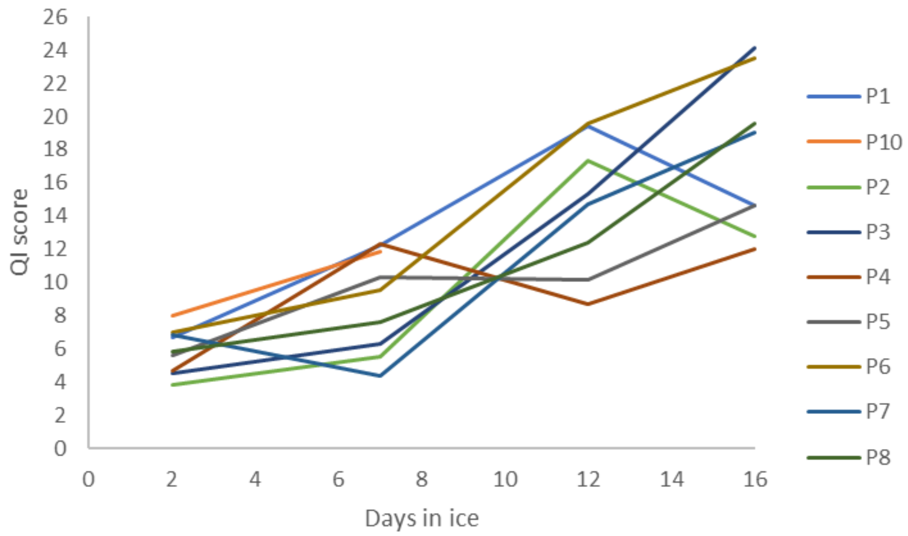


Figure 10: Quality Index (QI) scores per panellist against storage time in ice

Table 4: Preliminary QIM scheme developed for fresh Senegalese sole after the pre-observation

Quality parameters	Description	Points
Skin		
Spot on ocular side	Very clear, light	0
	Less clear	1
	Faded/unclear	2
Ocular side	Dark colour, bright, shiny appearance, blue/whitish spots evident. No discoloration	0
	Rather dull or pale, colour slightly faded or colour slightly paler somewhat shrunken skin	1
	Dull, pale, purple discolouration (seen at the edges of the the fin), shrunken skin evident. Slightly yellowish discoloration	2
	Pale, dull, yellowish shrunken skin evident	3
Blind side	Bright white, no discolouration/dark colour, bright shiny appearance (if no white area on blind side)	0
	Some purple discolouration at the edges of the fin	1
	Dull, purple, yellow discolouration at the fins and in the middle	2
Mucus	Abundant and almost clear/ translucent, not clotted	0
	Slightly clotted and milky	1
	Clotted and slightly yellow	2
	Clotted, yellowish and brownish, less mucus	3
Odour	Neutral	0
	Grassy, metallic	1
	Sour, fermented	2
	Rotten	3
Texture (Ocular side)	Very firm and stiff when lifted (in rigor)	0
	Firm, elastic	1
	Less firm, less elastic	2
	rather soft, reduced elasticity	3
Eyes		
Form	Flat eye, eye socket convex	0
	Slightly sunken, eye socket sunken	1
	Sunken and/ or swollen, eye socket shrunken	2
Clarity/brightness	black and clear pupil, golden rim around the pupil bluish/ whitish at the top of the eye	0
	Rather matte pupil, faint golden rim around the pupil	1
	Matte and milky pupil, pinkish/yellowish colour of the around the pupil	2
Gills		
Odour	Fresh or neutral	0
	Metallic, cucumber	1
	Grassy, slightly sour/fermented	2
	Rotten, sour, sulphurous	3
Colour	Dark purple/dark red	0
	Slightly faded colour	1
	Fades colour, slightly discoloured	2
	Yellowish, brownish discolouration	3
Mucus	Mucus almost clear and transparent	0
	Milky and slightly clotted	1
	Yellowish, thick, clotted	2
	Quality Index Total	**0-28**

Day 7: Abundant mucus, almost clear and not clotted.

Day 14: Mucus less and slightly clotted

Figure 11: Mucus on skin slightly clotted and less with storage time

Day 1: Bluish/whitish spots light and very clear.

Day 13: Bluish/whitish spots fades out and skin dull and pale

Figure 12: Appearance of Senegalese sole after 13 days of storage. Bluish/whitish spots on ocular side fades out with storage time.

	Day 7: slightly faded colour gills
	Day 14: gills colour fading (brownish discoloured)

Figure 13: Appearance of the gills with storage time in ice.

	Day 1: white area bright with no discoloration
	Day 13: slightly duller color with some purple discoloration at the edges of the fin

Figure 14: Appearance on the blind side shows skin lost the brownish pigmentation and became pale and purple discoloured.

Table 5: Quality Index Method (QIM) scheme developed for fresh Senegalese sole

Quality parameters	Description	Points
skin		
Spot on ocular side	Very clear, light	0
	Less clear	1
	Faded/unclear	2
Appearance Ocular side	Dark colour, bright, shiny appearance, no discoloration skin not shrunken	0
	Rather dull or pale, colour slightly faded or colour slightly Paler, somewhat shrunken skin	1
	Dull, pale, purple discolouration (mostly at the edges of the the fins), shrunken skin evident. Slightly yellowish discoloration	2
	Pale, dull, yellowish. shrunken skin evident	3
Mucus	Abundant and almost clear/translucent, not clotted	0
	Slightly clotted and milky. Slightly less mucus	1
	Clotted and slightly yellow. Mucus thicker and drier	2
	Clotted yellowish and brownish. Mucus thicker and almost dry	3
Odour	Neutral, weak	0
	Grassy, cucumber	1
	Slightly sour, dirty table cloth	2
	Sour, fermented, rotten	3
Appearance Blind side	White area: bright white, no discoloration Dark area: dark colour, bright shiny appearance	0
	Slightly duller, some purple discoloration at the edges of the fin	1
	Dull, purple, yellow discolouration at the fins and in the middle	2
Texture (Ocular side)	In rigor, very firm and stiff when lifted	0
	Firm, elastic	1
	Less firm, less elastic	2
Eyes		
Form	Flat eye, eye socket convex	0
	Eyes and eye socket slightly sunken	1
	Eye sunken and/ or swollen, eye socket sunken	2
clarity/brightness	Black and clear pupil, golden rim around the pupil, brown grey bluish iris	0
	Slightly matte pupil, faint golden rim around the pupil, Slightly pink iris	1
	Rather matte pupil, faded golden rim, pinkish/yellowish Colour of the iris	2
	Matte and milky pupil, faded golden rim, pinkish/yellow Colour of the iris	3
Gills		
Colour	Dark purple/dark red	0
	Slightly faded colour	1
	Fades colour, slightly discoloured	2
	Yellowish, brownish discolouration	3
Odour	Fresh or neutral	0
	Metallic, cucumber	1
	Grassy, slightly sour/fermented	2
	Rotten, sour, sulphurous	3
	Quality Index Total	**0-26**

5.2 Shelf life study of Senegalese sole

5.2.1 Temperature Profile

The experiment commenced on the 18th January 2019. The temperature of the control group boxes stored at 0^0C was 0-1 °C during the whole storage time as shown in Figure 15 (lower graph), while the temperature in the boxes holding the T-abused group increased from 0-1°C to 17°C in 16 hours, but decreased very rapidly upon re-icing (Figure 15, lower graph). Results shows that the temperature loggers placed to capture room temperature recorded and temperature between 0^0 C and 3^0 C. The outside temperature of the T-abused group was 20°C during the 16 hours (upper graph in figure 15).

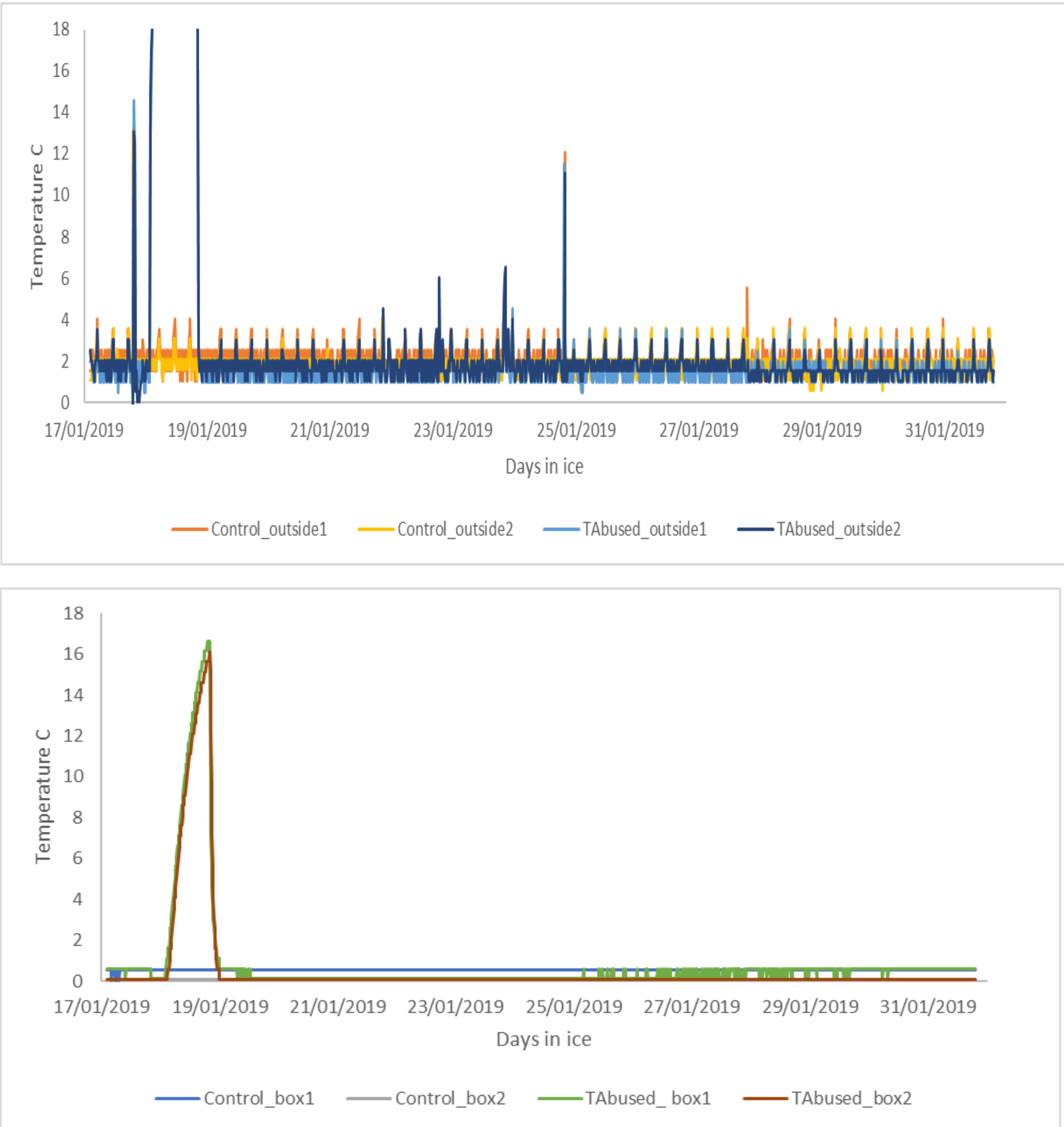


Figure 15 Temperature logger used to monitor the temperature of two groups of whole fresh Senegalese sole stored at different temperatures Control group (Control) and temperature abused group (T. Abused). The upper figure shows ambient temperature and lower figure shows temperature inside boxes during storage days.

5.2.2 *QIM results of whole fresh Senegalese sole*

The QI based on the average of the panelists was calculated for each storage day. All the selected parameters from the trial were useful in reflecting the quality changes of the Senegalese sole with respect to average QI scores with storage time in ice. A high correlation (R^2 = 0.9816) was found between QI and storage time in ice (Figure 16). There was a difference between the T-abused and control samples for days 12 and 16 where the abused has a slightly higher average with p-value of 0.003 for day 12 and p-value of 0.005 for day 16 Table 7. During the study, a QI variation was obtained by the panelists. Some panelists scored higher at the early storage days. The panelists were in more agreement at the last session of the evaluation (Appendix 5). The evaluation trend was similar for all panelists except for panelist four that scored lower (Figure 10). The correlation (r) between the average scores for each attribute and the storage time in ice (control and T-abused) for Senegalese sole is shown in table Table 6. The quality index of the whole fresh Senegalese sole during storage for control and T-abused can be seen (

Figure 17) showing higher QI scores obtained after temperature abused.

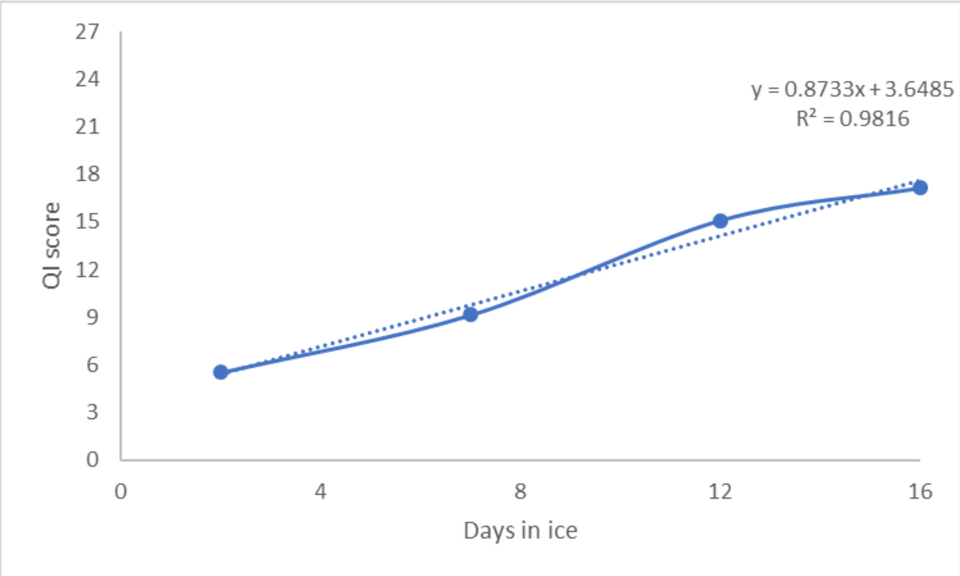


Figure 16: Correlation between Quality Index score of Senegalese sole stored in ice and storage days in ice

Table 6: Average scores of the Control and temperature abused group for each attribute assessed with QIM scheme method with storage time in ice for Senegalese sole. The range of scores of each attribute is demonstrated in parenthesis.

		Storage days (Control and T-abused)			
	Attribute	2	7	12	16
Skin	Spots on ocular side (0-2)	1.0	0.8	1.1	1.2
	Appearance ocular side (0-3)	0.5	0.8	1.4	1.6
	Mucus (0-3)	0.8	1.0	1.9	2.0
	Odour (0-3)	0.6	0.9	1.8	1.9
	Appearance Blind side (0-2)	0.4	0.9	1.3	1.6
	Texture ocular side (0-2)	1.0	1.1	1.5	1.5
Eye	Form (0-2)	0.2	0.8	1.2	1.6
	Clarity/brightness (0-3)	0.3	0.8	1.5	2.0

Gills	Colour (0-3)	0.2	1.0	1.4	1.7
	Odour (0-3)	0.5	1.1	2.0	2.3

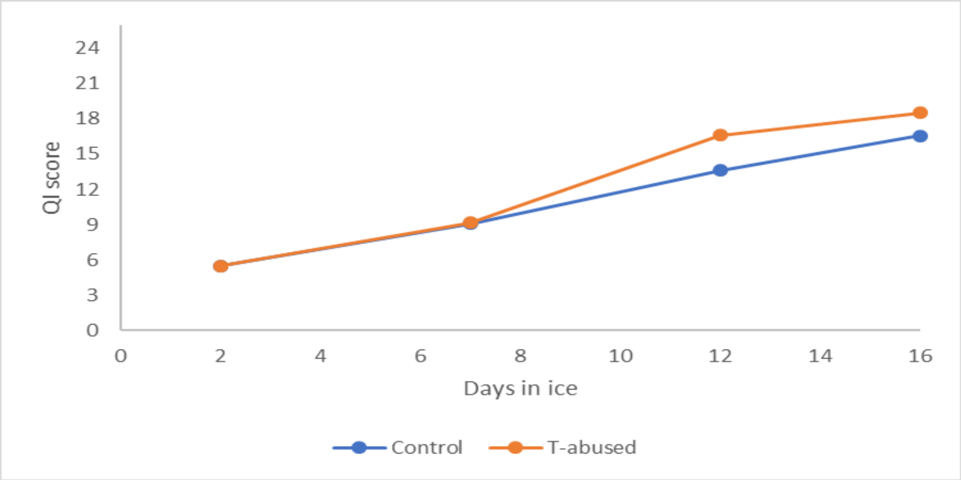


Figure 17: Quality Index score of Senegalese sole (Control and T-abused group) with storage days in ice

Table 7: Average QI scores of Senegalese sole (Control and T-abused) with storage days in ice

Days in ice	Control Group	Temperature Abused Group	p-value
D2	5.5	5.5	
D7	9.1	9.2	0.903
D12	13.6	16.6	0.003
D16	16.0	18.9	0.005

5.2.3 Torry Results of cooked Senegalese sole

According to sensory evaluation of cooked fish with Torry score sheet, the attributes which were perceived at the beginning of the storage described freshness characteristics Figure 18. Initially, scores were high for day 2 (7.8 average score for odour/flavour). For day 7, T-abused samples scored 8.7 on average and control temperature samples scored 8.3 (p = 0,081) **Error! Reference source not found.**. O n storage day 12, a decline of freshness was observed and an average score of 6.5 for the control samples and 5.7 for T-abused samples were recorded with a p-value of 0.171 (Appendices 6 and 7). Seven panellists evaluated all the samples on day 12 but only 3 tasted all samples. On day 16, six (6) panellists evaluated all samples but only one tasted all samples. There was a continuous loss of fresh odours of margarine, butter and flavour of slightly bitter, shellfish, and meaty at the early storage days until the cooked samples became insipid towards off flavour, rancid and metallic with slight sour odour by storage day 12. The spoilage attributes of bitter, traces of TMA and spoiled cheese were detected during the sixteenth day of storage when it reaches sensory rejection with storage time. From this it can be assumed that the shelf life of the fish is between 12 and 16 days when stored on ice. However, only three out of seven panellists tasted all samples on day 12, which indicates big differences between individual fish in the level of spoilage. In practice, the shelf life of each batch of fish is limited by the shortest shelf life in each batch, since if one fish is spoiled, the whole batch would be spoiled. The shortest shelf life could therefore be shorter than 12 days, given the handling conditions used in this experiment.

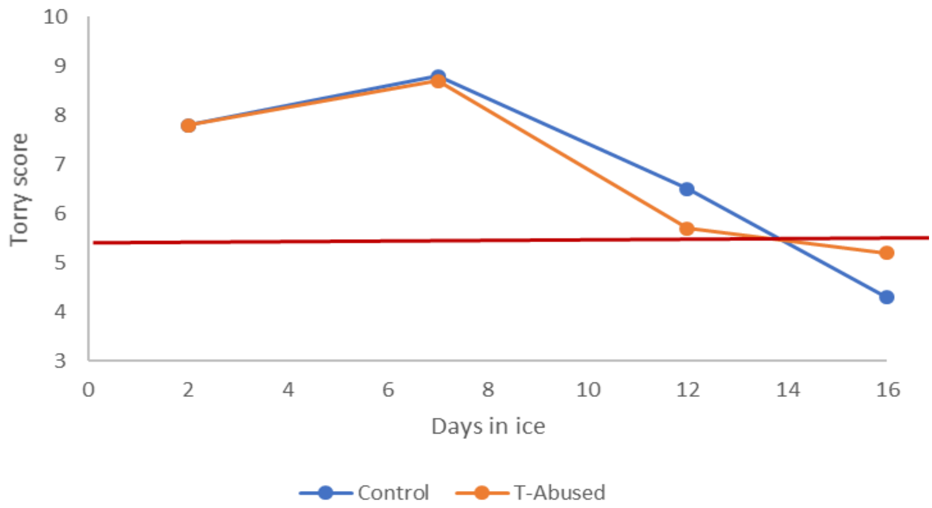


Figure 18: Average scores of odour/flavour and storage days in ice of cooked Senegalese sole (Control and T-abused group)

5.3 Chemical and Microbial Results

5.3.1 Chemical and fat content results

The formation of volatile bases (TVB-N) in the fillets of Senegalese sole increase in all samples Figure 19. Measurement of TVB-N fluctuated slightly throughout the storage period between 16.3 and 18.05 mgN/100g in both groups (control at 0^0 C and temperature abused). On all occasions lower numbers were obtained from both groups. The levels of TVB-N were under the limits of TVB-N for of 25-35 mgN/100g for some fish species (Dalgaard, 2000). TVB-N value for T-abused appeared to be higher than the control. Senegalese sole is considered a lean fish with low fat contents (less than 6%). In the present study, the lipid contents varied from 1.6-3.1% (Borges et al., 2009).

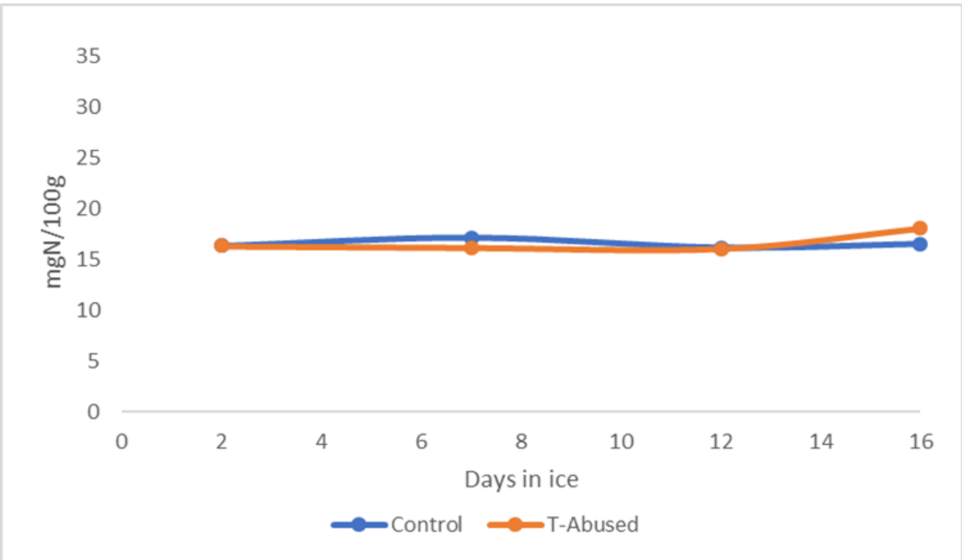


Figure 19: Changes in TVB-N values in Senegalese sole stored in ice (Control and T-abused group)

5.3.2 Microbial Results

The microbial values obtained during storage for both groups (control and T-abused) show an increase with storage time in ice with initial values higher in the control samples at day 7 (Figure 20).The values ranged between 3.62 and 4.82 log_{10} cfu/g when the cooked fish was rejected by the panel. The proportion of the SSOs counts (H_2S-producing bacteria) of the TVC were the lowest at the beginning of storage but increased with storage time (Figure 20). After 16 days of storage, the TVC and H_2S were 7 log_{10} cfu/g and 6 log_{10} cfu/g respectively. On all occasions, lower numbers were obtained from both groups (control and T-abused). *Shewanella putrefaciens* and *Pseudomonas spp*., the specific spoilage bacteria, normally predominates within this group and it has been reported that these organisms are able to produce off-odours in fish products (Dalgaard, 2000). The TVC were lower in the control fish compared to the T-abused with storage days. A cell concentration of more than 8 log_{10} cfu/g are normally required to cause spoilage of ice-stored fish (Gram & Huss, 1996).

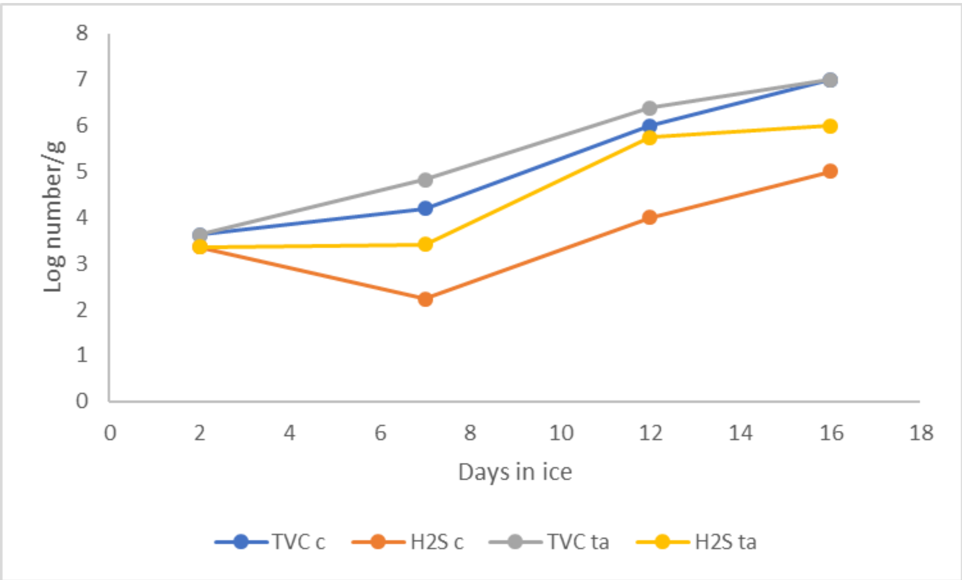


Figure 20: Changes of Specific Spoilage Organisms (H_2S-producing bacteria) and total viable count (TVC) on Senegalese sole stored on ice for 16 days (Control and T-abused group)

6 DISCUSSION

A modified version of QIM scheme for Senegalese sole was suggested based on previous QIM version of (Gonçalves et al., 2007) for Senegalese sole, and the QIM scheme for sole (Martinsdottir et al., 2001). During the development, a pre-study was done to monitor sensory changes of whole raw Senegalese sole with the aim of evaluating the sensory schemes. Following some modifications, the scheme was finalised parallel to panel training. The scheme was then evaluated in a full-scale shelf life study, where the effect of short temperature abuse was evaluated. The fish used in the pretrial and the main shelf life study received different treatment after slaughtering the fish, as the fish used in the pretrial was cooled in ice slurry, whereas the fish in the main shelf life study was iced after slaughtered. It can be assumed that the fish cooled down faster in the ice slurry.

6.1 Development of QIM scheme

During the pre-observation, fish were acceptable following storage for up 21 days. Strong odours like milk jug and traces of TMA were evident at storage day 21 when the panel decided not to evaluate the cooked fish. The final QIM scheme for Senegalese sole consists of 10 parameters (Table 5), resulting in a total of 26 demerit points. The scores for quality attributes in the QIM scheme increased differently with storage time in ice, but a linear relationship with high correlation was found between QI and storage time in ice.

6.2 Shelf life study

6.2.1 Sensory evaluation of fresh whole fish (QIM)

The final QIM scheme for Senegalese sole consisted of 10 parameters and 26 demerit points (*Table 5*). There was significant linear relationship ($R^2 = 0.9816$) between the average QI and storage time in ice, which was a stronger linear relationship than previously reported for the QIM scheme published by (Gonçalves et al., 2007) for Senegalese sole.

Changes in sensory attributes of both groups (Control and Temperature abused) indicated that the Senegalese sole was approaching the end of shelf-life when increasingly characterised by purple/yellowish discoloration at the fins and in the middle of the blind side and clotted and slightly yellow mucus after 16 days of storage in ice. At this time, the average QI was 16.5 for the Control group and 18.5 for the Temperature abused group. Sensory rejection of the Senegalese sole was considered to occur approximately after 12- 16 days in ice. There was a difference between the control group and the T-abused group on storage days 12 and 16, where the T-abused group received slightly higher average scores. This indicates that the temperature abuse resulted in somewhat shorter shelf life of the Senegal sole.

There was abundant mucus on the surface of the fish in the pretrial, and less mucus in the shelf life study. This could be the different treatment after slaughtering the fish, as the fish used in the pretrial was cooled in ice slurry, whereas the fish in the main shelf life study was iced after slaughtering. It can be assumed that the fish cooled down faster in the ice slurry.

6.2.2 Torry score in shelf life study

A gradual decrease of the Torry scores was observed during storage time. End of shelf life is usually determined when sensory attributes related to spoilage such as traces of TMA, slightly sour, rancid, bitter flavour and spoiled cheese, sour beer odours become evident. Most panelists detected rancidity and off 'flavours' of some samples on storage day 12 (average score: 5.7 for temperature abused group and 6.5 for control group). The limits for acceptable consumption have been reported to be below 5.5 on the scale 10 to 3 according to (Martinsdottir et al., 2001). According to this, both groups were no longer of acceptable consumption quality on storage day 16, where both were below these limits.

Therefore, the shelf life of the Senegalese sole in this study was evaluated between 12 and 16 days in ice, both for the control and temperature abused group and there was no significant difference between the two groups which indicates that the temperature abuse did not have a big effect on the shelf life of the fish. The results indicate individual differences in spoilage level on day 12 with some fish more spoilt than others. Therefore, in practice the shelf life could be shorter than 12 days given that the whole fish batch would be considered spoiled if only a few fish have developed spoilage characteristics. (Gonçalves et al., 2007) reported a maximum shelf life for Senegalese sole to be 15 days in ice, which is similar to the results in our study. However, the 12-16 days is considerably shorter than what was expected based on the results in the pretrial (20-21 days). The different treatments after slaughtering, slurry ice for the pretrial and ice for the shelf life study, could explain longer shelf life of the fish used in the pretrial. Generally, the texture was rather soft, juicy and tender (Appendices 8 &9).

6.3 Chemical and microbial counts

The levels of TVB-N ranged from 15.5 to18.7 mgN/100g during the storage time for both groups, with a slight upwards trend for the temperature abused group on storage day 16 (Figure 19). The levels of TVB-N were under the limits of TVB-N for of 25-35 mgN/100g for some fish species (Dalgaard, 2000). It can therefore be concluded that TVN measurements at the time of sensory rejection cannot be the cause of spoilage for Senegalese sole.

The proportion of the SSOs counts and the TVC were the lowest at the beginning of storage but increased with storage time (*Figure 20*). After 16 days of storage, the TVC were 7 $\log_{10}$ cfu/g for control and 7 $\log_{10}$ cfu/g for T-abused and SSO were 5 $\log_{10}$ cfu/g for control and 6 $\log_{10}$ cfu/g for T-abused respectively. Comparing H_2S-producing bacteria the results obtained in this work with the results on Senegalese sole by (Tejada & De las Heras, 2007) it can be seen that numbers of H_2S producing bacteria increased in one of the lot from the beginning of storage reaching a level of 8 $\log_{10}$ cfu/g at day 18.

A cell concentration of more than 8 $\log_{10}$ cfu/g are normally required to cause spoilage of ice-stored fish (Gram & Huss, 1996). The total viable counts and H_2S – producing bacteria values at the end of storage time did not reach very high values. Thus, the microbiological storage life of the fish was in good agreement with the results of the sensory evaluation. The dominant microflora on temperate waters are mostly *Pseudomonas* and *Shewanella*. Only a very limited number of bacteria invade the flesh during ice storage (Huss, 1995).

7 CONCLUSION AND RECOMMENDATIONS

A QIM scheme for fresh Senegalese sole (*Solae senegalensis*) was developed during this study. The QIM developed consists of 10 parameters grouped in three main categories, resulting in a total of 26 demerit points. The scores for quality attributes included in the QIM scheme increased differently with storage time in ice, but a linear relationship with high correlation was found between QI and storage time in ice. The QIM results indicate that exposure to higher temperatures (20°C) for 16 hours results in faster spoilage and shorter shelf life of the fish.

In this work Senegalese sole was stored in different storage conditions; a steady 0°C in ice and a group stored at 20 °C for 16 hours followed by ice storage at 0°C. Storage at a 20°C for a relatively short time caused somewhat more rapid spoilage than the continuous storage at low temperatures. The temperature in Sierra Leone is even higher with an average range of 24^0C to 30^0C, and this high ambient temperature accelerates fish spoilage even more. Fish can be kept for a short period therefore it must be immediately preserved to delay spoilage and to extend the shelf life. Improved handling, storage temperature, processing techniques and packaging are key factors in delaying fish spoilage in Sierra Leone. Handling should be hygienic and under controlled temperature, otherwise spoilage might increase very rapidly. Fresh fish should immediately be stored at appropriate conditions and should not be exposed to ambient temperature even for a short period of time. Fish should be chilled rapidly after catch and stored at low temperatures to obtain as long shelf-life as possible.

The main limiting factor for length of shelf life was mainly due to sensory attributes describing spoilage. Hence the chemical (TVB-N) parameter is not suggested as a reliable indicator for freshness quality of iced Senegalese sole. The total viable counts (TVC) increased to 7 log10 cfu/g in both groups during the storage time. The specific spoilage bacteria (SSOs) reached 6 $\log_{10}$ cfu/g in the Temperature abused group but were only 5 $\log_{10}$ cfu/g in the Control group at the end of the storage time. SSOs are responsible for off-odours and flavours in fish (Dalgaard, 2000). Therefore, the higher numbers of SSOs in the Temperature abused group support the sensory results indicating a shorter shelf life of the Temperature abused group.

The results indicate that the fish from the pretrial had somewhat longer shelf life than the fish used in the shelf life study. This could be caused by the different treatments after slaughtering the fish, as the fish used in the pretrial was cooled in ice slurry, whereas the fish in the main shelf life study was iced after slaughtering. It can be assumed that the fish cooled down faster in the ice slurry.

Therefore, the activity of these organisms can be controlled, reduced or even retarded by proper handling and immediate lowering of the temperature.

It is worth emphasising the importance of following a cold chain to increase the storage life of fish and fish products and in maintaining freshness and quality. Similar studies should be performed in Sierra Leone to establish appropriate quality index measurement (QIM) for Sierra Leonean species for freshness sensory assessment.

QIM appears to be an easy, rapid and efficient tool to assess the history and estimate the remaining shelf life of fish products. This method is suitable for freshness evaluation of fish in the fish industry and at landing sites. Therefore, the implementation of this tool would help in determining how handling, processing and storage conditions affect the fish shelf life. Fishermen should be trained on the importance of using ice during handling and storage of fish in order to extend the storage life of fish.

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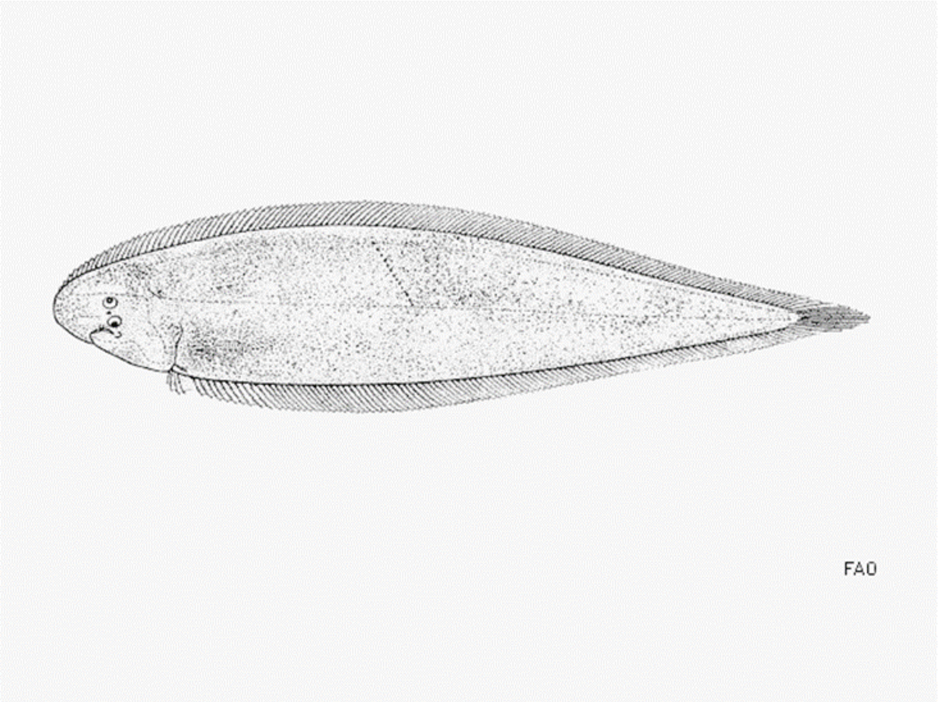
APPENDICES

Appendix 1: Scientific and local names of some commercially exploited fish species in Sierra Leone

Family	Scientific Name	Local Name
Clupeidae	Ethmalosa fimbriata	Bonga
	Illisha africana	Lati
	Sardinella maderensis	Flat herring
	Sardinella aurita	Round herring
Carangidae	Chloroscombrus chrysurus	Atlantic bumper
	Trachurus tracae	Horse mackerel
Sciaenidae	Pseudotolithus senegalensis	Cassava croakers
	Pseudotolithus elongatus	Lady
Polynemidae	Pentanemus quinquarius	Royal threadfin
	Galeiodes_decadactylus	Shinenose
Sparidae	Dentex spp.	Snapper
	Sparus spp.	Bluespotted seabream
Penaeid	Penaeus notialis	Pink shrimps
Ballistae	Balistes	

Appendix 2: *Cynoglossus senegalensis* (Tongue sole)



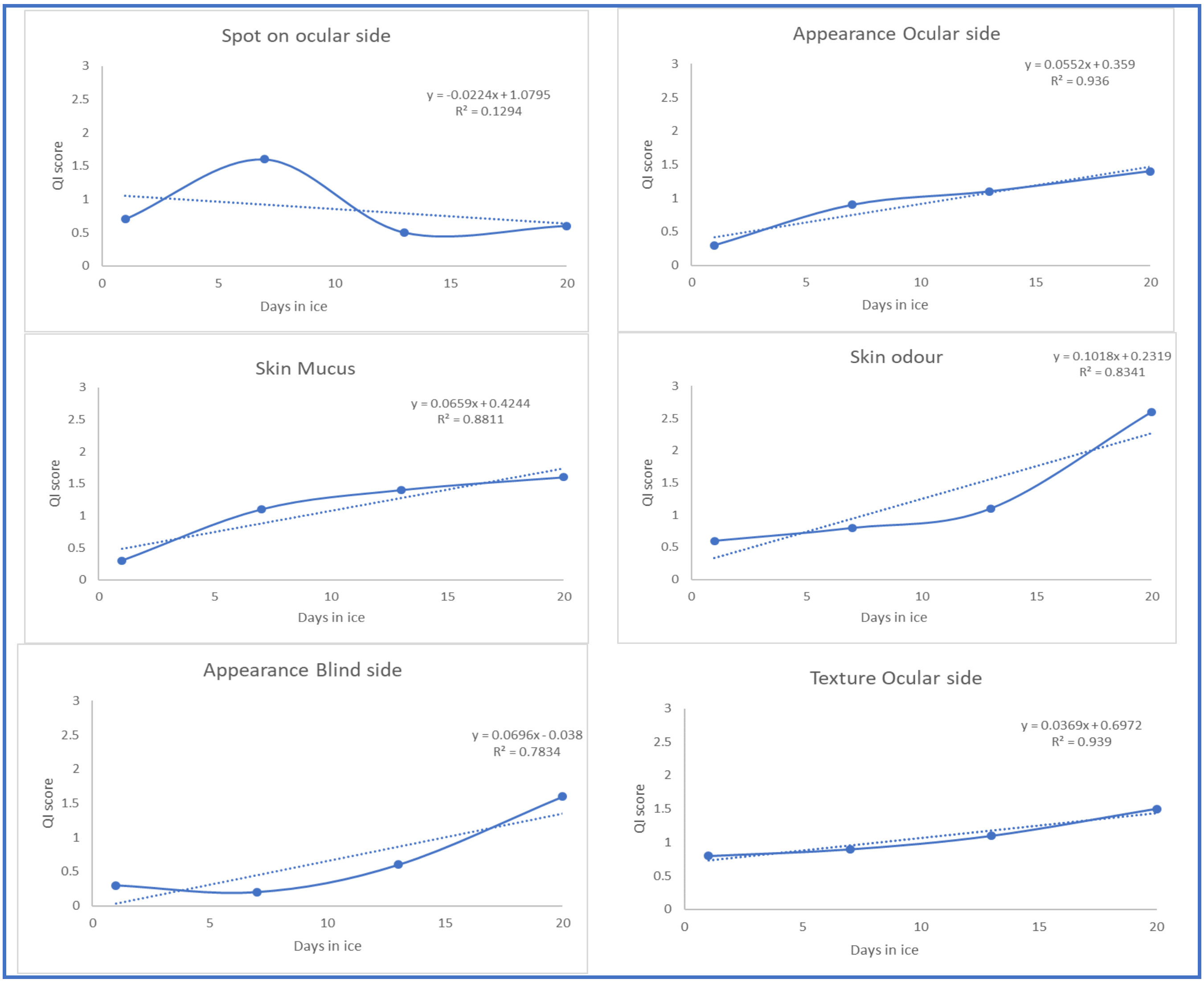
Source: FishBase 2018 (Photo: FAO)

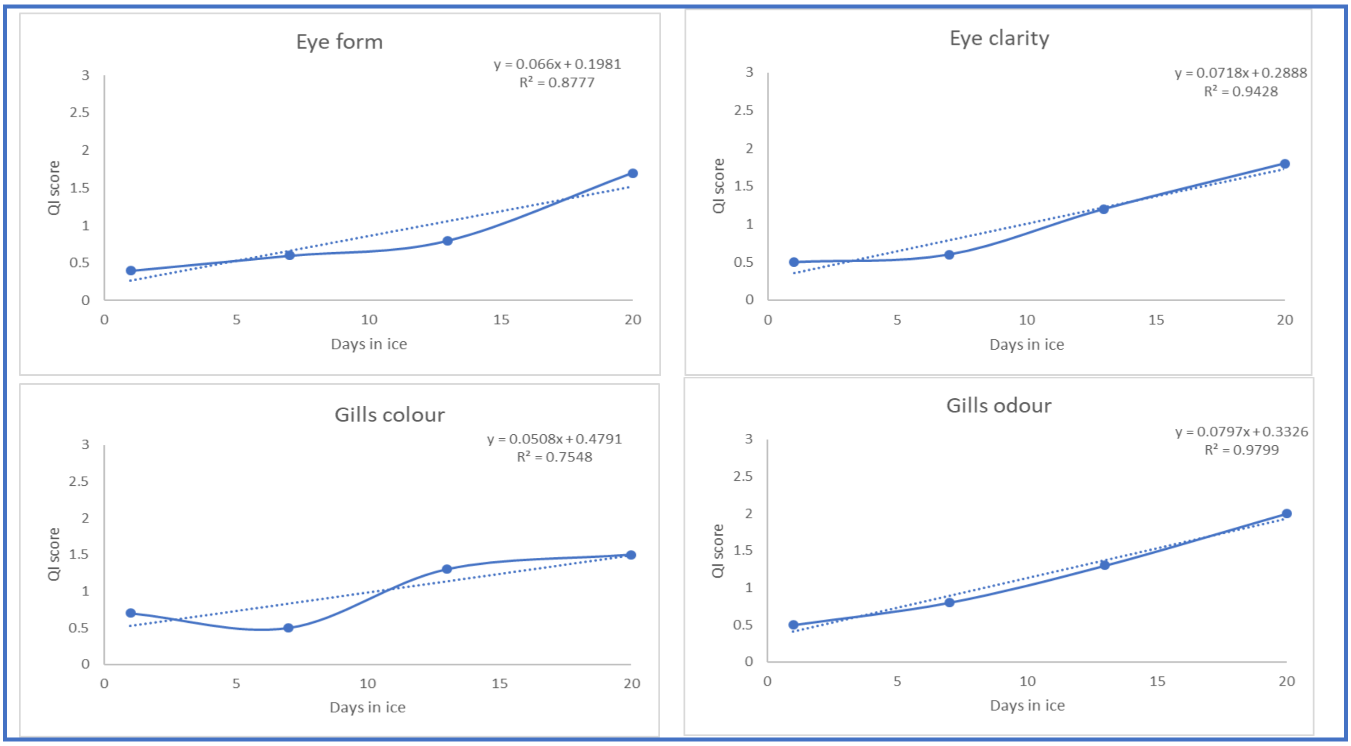
Appendix 3: Initial Quality Index Method (QIM) for freshness Assessment of whole Raw Farmed Senegalese Sole and the QIM scheme proposed for Whole Raw Farmed Senegalese Sole

Quality parameter		Descriptors/demerit points according to (Gonçalves et al., 2007)	Score	Descriptions based on pre-observation	score
Skin Appearance				Very clear, light	0
				Less clear	1
				Faded / unclear	2
	Ocular side	Fresh, bright, no discolouration	0	Dark colour, bright shiny appearance. No discolouration	0
		Rather dull or pale, somewhat shrunken skin	1	Rather dull or pale, colour slightly faded or colour slightly paler, somewhat shrunken skin	1
		Dull, pale, some green or purple discoloration	2	Dull, pale, purple discoloration (seen at the edges of the fins), somewhat shrunken skin evident	2
		Dull, green and purple discolouration, very shrunken skin	3	Pale, dull, yellowish. Shrunken skin evident	3
	Blind side	bright white	0	Bright white, no discoloration / dark colour, bright shiny appearance (if no white area on blind side)	0
		some purple discoloration at the edges of the fins	1	some purple discoloration at the edges of the fins	1
		Dull, purple yellow discoloration at fins and in the middle	2	Dull, purple, yellow discoloration at fins and in the middle	2
		Yellow and purple discoloration	3		
	Mucus	Clear, not clotted	0	Abundant and almost clear/ translucent, not clotted	0
		Slightly clotted and milk	1	Slightly clotted and milky	1
		Clotted and slightly yellow	2	Clotted and slightly yellow	2
		Yellow and clotted	3	Clotted, yellowish and brownish, less mucus	3
	Odour		0	Neutral	0
			1	Grassy, metallic	1
			2	Sour, fermented	2
			3	Rotten	3
Texture (ocular side)		Firm, elastic	0	Very firm and stiff when lifted (In-rigor)	0
		Less firm, elasticity	1	Firm, elastic	1
		Soft	2	Less firm, less elastic	2
		Very soft	3	Rather soft, reduced elasticity	3
Eyes	form	Flat, eye socket convex	0	Flat eye, eye socket convex	0
		Slightly sunken, eye socket shrunken	1	Slightly sunken, eye socket sunken	1
		Sunken and/or swollen, eye socket shrunken	2	Sunken and/ or swollen, eye socket sunken	2
	Brightness	Black and clear pupil, golden rim around the pupil	0	Black and clear pupil, golden rim around the pupil Bluish/whitish at the top of the eye	0
		Rather matte, faint golden rim around the pupil	1	Rather matte pupil, faint golden rim around the pupil	1
		Matte, purple/reddish	2	Matte and milky pupil, pinkish/yellowish colour of the rim around the pupil	2

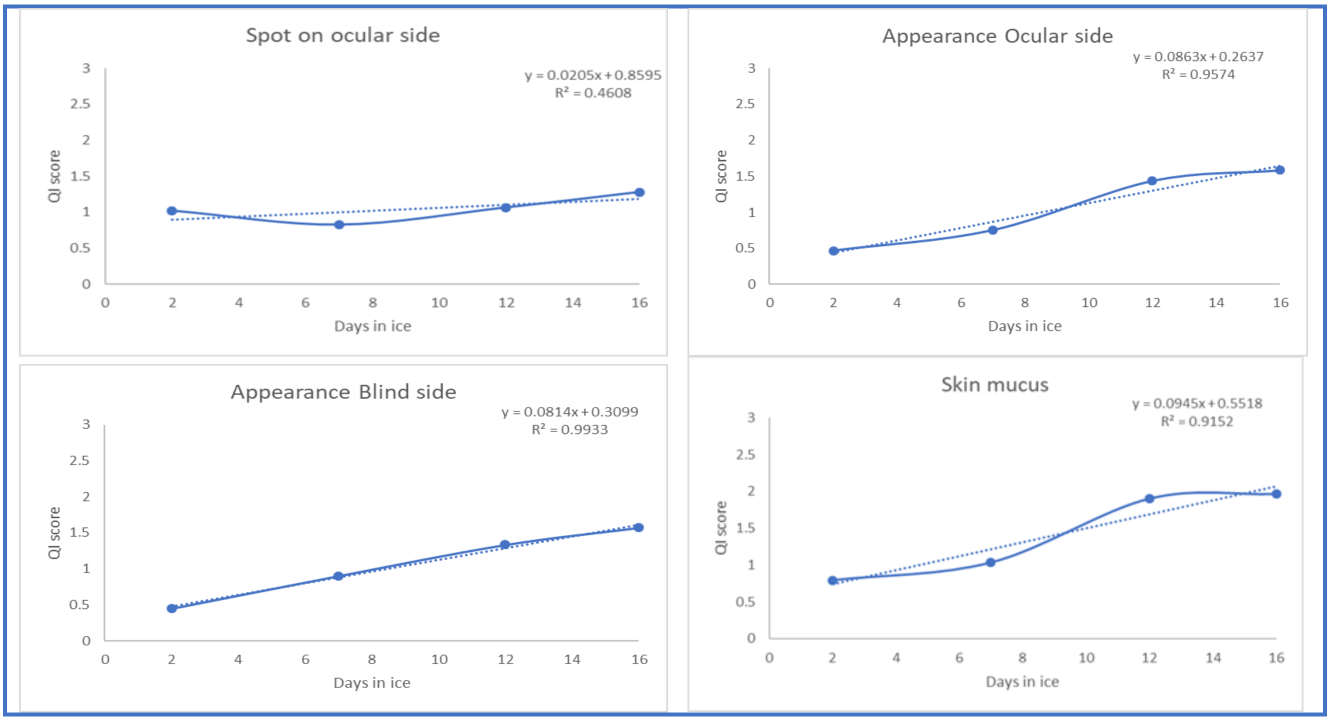
Gills	Odour	Fresh, seaweedy	0	Fresh or neutral	0
		Neutral, metallic, rubbery	1	Metallic, cucumber	1
		Musty, sour	2	Grassy, slightly sour / fermented	2
		Rotten, sour, sulfurous	3	Rotten, sour, sulfurous	3
	Colour	Bright, light red	0	Dark purple/dark red	0
		Slightly discoloured	1	Slightly faded colour, gill slightly yellow near the gills	1
		Discoloured	2	Faded colour, slightly discoloured	2
		Yellowish, green/blue discolouration	3	Yellowish, brownish discoloration	3
	Mucus	clear	0	Mucus almost clear and transparent	0
		milky, slightly clotted	1	Milky and slightly clotted	1
		yellow, thick, clotted	2	Yellowish, thick, clotted	2
Flesh	**Mucus**	No mucus	0		
		Clear	1		
		Milky, slightly clotted	2		
		Yellow, thick. Clotted	3		
	Colour	Translucent, bluish	0		
		Slightly yellowish	1		
		Yellow, discoloured	2		
		Waxy, opaque, yellow	3		
Quality index			**(0-28)**		**(028)**

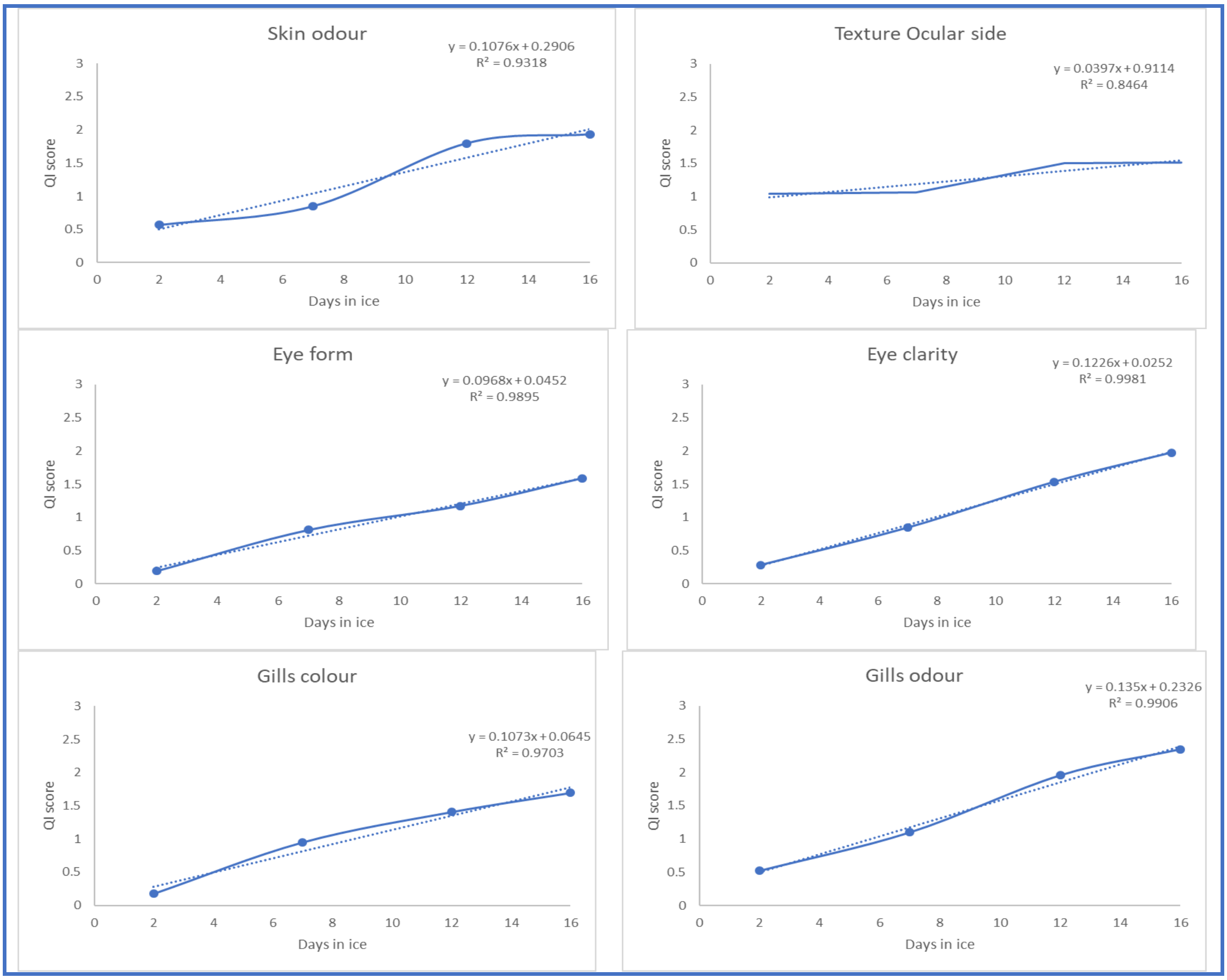
Appendix 4: Average scores versus storage days during the training for each attribute evaluated (QIM)





Appendix 5: Average scores versus storage days for the shelf life study for each attribute (QIM)





Appendix 6: Torry score sheet for texture evaluation for cooked Senegalese sole

Torry score		
Soft	Firm//soft	Softness in first bite
Juicy	Dry//juicy	Dry: draw liquid from mouth. Juicy: releases liquid when chewed
Tender	Tough//tender	Tenderness when chewed
Mushy	None//much	Mushy texture when chewing. Much: skyhumar (mushy langoustine)
Sticky	None//much	Stickiness, glues teeth together when biting the fish

Appendix 7: Average values for Torry score (flavour, odour and texture) for coked Senegalese sole for storage temperature (Control and T-abused)

Days in ice	Torry Score	Soft	Juicy	Tender	Mushy	Sticky
2						
	7.8	58	49	63	40	56
7						
ZERO	8.3	64	61	69	36	39
ABUSED	8.7	60	63	71	32	37
p-value	0.081	0.339	0.610	0.566	0.398	0.693
12						
ZERO	6.5	71	62	65	49	37
ABUSED	5.7	73	71	68	52	30
p-value	0.171	0.742	0.365	0.706	0.649	0.047
16						
ZERO	4.3					
ABUSED	5.2					
p-value	0.237					

Appendix 8: Photos showing the sensory evaluation of Senegalese sole during pre-observation by QIM scheme and Torry methods.