Final project 2017



unuftp.is

TRANSFER OF SENSORY METHODOLOGY TO ARTISANAL FISHERIES BY DEVELOPMENT AND APPLICATION OF QUALITY INDEX METHOD (QIM)

Ritha H. J. Mlingi Kigoma Regional Secretariat, Tanzania rithajomy@gmail.com

> Supervisor: Kolbrún Sveinsdóttir Matís kolbrun@matis.is

ABSTRACT

The aim of this project was to learn how sensory evaluation may be applied in Tanzanian fisheries through the development and application of quality index method (QIM) scheme for thawed red fish fillets and pre-observation of sensory changes in whole thawed herring. After thawing, both redfish fillets and whole herring were stored in cold room at 1.7 ± 1.5 °C up to 14 and 18 days respectively. A QIM scheme for thawed redfish fillets to evaluate shelf life was proposed. A strong correlation between the Quality Index (QI) and storage time was found. The calculated QI increased linearly with storage time at 1.7 ± 1.5 °C (QI= 0.5444 x (days in cold storage) + 2.907, R² = 0.8156). Generic descriptive analysis (GDA), total viable counts (TVC) and counts of H₂S-producing bacteria were done on redfish fillets only. The maximum storage time of thawed redfish fillets stored at 1.7 ± 1.5 °C was estimated 9 days based on GDA, TVC H₂S-producing bacteria counts. A draft QIM scheme for thawed whole herring was proposed. The knowledge and experience obtained from the project would be implemented in Tanzanian fisheries as QIM methodology is a good basis for the training of fisheries professionals.

Keywords: Redfish; Herring; Sensory evaluation; Quality Index Method; Shelf life.

This paper should be cited as:

Mlingi, R. 2018. *Transfer of sensory methodology to artisanal fisheries by development and application of quality index method (QIM)*. United Nations University Fisheries Training Programme, Iceland. Final project. http://www.unuftp.is/static/fellows/document/Ritha17prf.pdf

TABLE OF CONTENTS

1	INT	TRODUCTION					
2	LIT	LITERATURE REVIEW					
	2.1	Atla	antic redfish (Sebastes marinus)	.6			
	2.2	Atla	antic herring (Clupea harengus)	.7			
	2.3	She	lf life of fish	.7			
	2.4	Sen	sory evaluation of fish	.8			
	2.4.	1	Quality Index Method (QIM) Scheme	.8			
	2.4.	2	Descriptive analysis	.9			
	2.5	Mic	crobial analysis	10			
3	MA	TER	RIAL AND METHODS	11			
	3.1	Exp	perimental design	11			
	3.1.	1	Proposed QIM scheme for thawed redfish fillets	11			
	3.1.	2	Proposed preliminary scheme for thawed whole herring (Clupea harengus)	12			
	3.2	Sen	sory evaluation of thawed red fish fillets using Quality Index Method (QIM)	12			
	3.2.	1	Development of QIM scheme for thawed red fish fillets	13			
	3.2.	2	Evaluation of freshness using QIM scheme in a shelf life study	13			
	3.3	Sen	sory evaluation of cooked fillets using GDA	14			
	3.3.	1	Training on GDA	14			
	3.3.	2	Evaluation of shelf life using GDA	14			
	3.3.	3	Microbial counts	14			
	3.4	Pre	-observation of sensory changes in whole thawed herring	15			
4	RES	SUL	TS	15			
	4.1	Ten	nperature	15			
	4.2	Dev	velopment of QIM scheme for thawed redfish fillet	16			
	4.3	She	If life study	18			
	4.3.	1	Evaluation of raw fillets using QIM	18			
	4.3.	2	Evaluation of cooked fillets using GDA	23			
	4.3.	3	Microbial counts	27			
	4.4	Pre	-observation of sensory changes in whole thawed herring	27			
5	DIS	CUS	SSION	30			
	5.1	Dev	velopment of the scheme	30			
	5.2	She	If life study	30			
5.2.1 Evaluation of raw fillets using QIM							

	5.2.2	Evaluation of cooked fillets using GDA	31
	5.2.3	Microbial counts	31
6	CONCI	LUSION AND RECOMMENDATION	32
7	ACKN	OWLEDGEMENTS	32
8	LIST O	F REFERENCES	33
9	APPEN	IDIX	36

LIST OF FIGURES

Figure 1. Atlantic Redfish (Sebastes marinus)
Figure 2. Atlantic Herring (<i>Clupea harengus</i>)7
Figure 3 Experimental design for the development and application of QIM scheme for Red fish
(Sebastes marinus) fillets stored at 0-4°C
Figure 4. Temperature loggers A (I Button temperature logger (DS1922L/T) and B (Onset
UTBI-001 Tidbit v2 temperature logger) installed at -25°C and 0-4°C
Figure 5. Experimental design for QIM application to pre-observation of thawed whole herring
stored at 0-4°C for up to 18 days.
Figure 6. Fillets prepared for QIM evaluation displaying back bone side(left) and skin side
(right)
Figure 7. Preparation of cooked samples for GDA14
Figure 8. a) Temperature log of frozen storage of redfish fillets outside and inside the box at -
$25 {}^{0}$ C and b) thawing storage room temperature (1.7±1.5 °C)
Figure 9. Fillets with different storage time
Figure 10. Average score for each quality attribute evaluated with QIM scheme for thawed
redfish (Sebastes marinus) fillets
Figure 11. Quality Index of redfish fillets over storage days 11a (with all attributes), 11b
(without gaping and texture attributes)
Figure 12. Distribution of quality index score of each red fish fillet with each storage day.
(numbers and letters differentiate 3 fillets that were evaluated for each storage day but fillets
with 1 day of storage were evaluated twice in different days)
Figure 13. Quality index score of different panellists with storage time23
Figure 14. Freshness (a) and spoilage indicating (b) odour attributes score of cooked red fish
fillets against storage days
Figure 15. Freshness (a) and spoilage indicating (b) flavour attributes score of cooked red fish
fillets against storage days
Figure 16. Appearance (a) and Texture (b) attributes score of cooked red fish fillets against
storage days27
Figure 17. Total viable counts and counts of H2S-producing bacteria in red fish fillets against
storage days
Figure 18. Sensory changes in appearance of thawed herring from storage days 1,12 to 1829

LIST OF TABLES

Table 1. Quality Index Method scheme developed for thawed redfish (Sebastes marinus) fillets
Table 2. Distribution of quality index (QI) mean value and standard deviation of each red fish
fillet with each storage day
Table 3. Sensory Attributes of cooked red fish fillets samples assessed by GDA method.
Freshness attributes are marked with (+), spoilage indicating attributes with (-)24
Table 4. Mean sensory scores for attributes of cooked red fish fillets. Different letters (a, b, c)
indicate significant different values between samples within a line25

1 INTRODUCTION

Tanzanian fisheries are mainly artisanal with few commercial or industrial vessels. The fisheries depend mainly on major lakes (Victoria, Tanganyika, Nyasa), dams and small lakes, minor rivers and the small-scale marine fisheries near the shore of the Indian Ocean. The artisanal fishery uses a variety of fishing vessels, including boats with outboard engines, inboard engines and non-engine boats. The sector supports employment for more than 4 million people directly and indirectly working as fishers, traders, processors and suppliers. The national fish production is 363,000 metric tons of which the inland capture fisheries contributes 85 %. Total fish production contributes about 2% of the GDP (Fisheries, 2015).

Lake Tanganyika, in western Tanzania in the Albertine Rift, is the second deepest lake in world. The lake has more than 250 cichlids species, but only four Centropomid species which include *Lates stappersi* and two sardine species (*Stolothrissa tanganicae* and *Limnothrissa miodon*) which are of major commercial importance. Industrial fisheries on the lake collapsed in the 1990s, and now the main source of economic activity for the lake shore community is artisanal fisheries providing employment to more than 100,000 people from around 800 landing sites along the lake. Pelagic fish contribute to 20-40% of the protein in the diet of about one million people living around the lake.

The Lake Tanganyika artisanal fisheries faces challenges in onboard handling of fish. Boats lack chilling facilities, hence immediate processing at the landing site is very important to diminish deterioration and to keep the nutritional value as fresh fish sustains for a maximum of one day un-chilled. Generally, local knowledge on how to determine fish freshness is lacking at landing sites, fish markets, auctions, and storage facilities (cold storage). Training of personnel and application of relevant sensory evaluation methods is therefore needed.

There are different sensory evaluation methods of whole fish and fillets available, including the European Union (EU) Scheme, the Torry scheme and the QIM scheme. EU Scheme is the method most used and recommended for quality assessment of raw fish in the industry and the inspection service (Council Regulation (EC) No 2406/96 of November 26, 1996), but it is not species specific. Torry-scale is commonly used in the fish industries of some countries and by buyers of fresh fish. It only evaluates overall freshness, but not specific sensory attributes. The Quality Index Method (QIM) is inexpensive because no equipment is required, only trained assessors are needed. The method is quick, reliable and does not affect the integrity of the fish. Therefore, it can be used as the ideal tool for quality control management of fish in developing countries where application of more sophisticated methods is not possible. Generic descriptive analysis (GDA) and qualitative descriptive analysis (QDA) are used to evaluate cooked samples. GDA combines different approaches from all descriptive methods. GDA is frequently employed during practical applications to meet specific project objectives and is based on similar principles as the QDA method. GDA is a useful addition to sensory methods for determination of shelf life of fish based on taste.

The end of shelf life is mostly due to bacterial growth and is reflected in unpleasant sensory characteristics. Most seafood spoilage bacteria are characterised by their ability to produce H_2S and reduce trimethylamine oxide (TMAO). The high counts of H_2S -producing bacteria are associated with the rejection of several fish species, irrespective of the temperature and atmosphere air (Capell, 1997). Therefore, microbial analysis methods can be used to compare and verify the sensory analysis methods using microbial counts (total viable counts and H_2S -producing bacteria) and to estimate the remaining shelf life of the fish.

The primary aim of this project is to explore sensory evaluation methods which may be suitable for use on Lake Tanganyika, including training of sensory evaluation panelists. The sensory evaluation methods are important in evaluation of quality parameters and the condition of fish and fish products related to storage conditions and shelf life. Sensory methods can be relatively fast to apply, but there is lack of knowledge and training to apply such methods, hence the training of fisheries personnel in evaluation of fish quality and freshness is needed. Therefore, the development of QIM and interpretation of its results in relation to other sensory methods (GDA and microbial analysis), can be a simple and reliable tool for determination of freshness and estimation of the shelf life of fish. Awareness is needed on freshness quality of fresh fish commercially, its perishability proportional to storage temperature and storage time.

This sensory research study setup is based on fish handling temperature conditions (thawed) as in Tanzanian fisheries and uses two different fish species as test models. The first species (redfish) as fillets to learn how QIM procedures can be applied to various fresh fishery products and quality grading of raw material for split and whole dried fish products. The second species (herring) has similar characteristics as Tanzanian commercial pelagic fatty species such as coastal sardine and gives additional knowledge on application of QIM procedures to observe sensory changes in whole fish.

2 LITERATURE REVIEW

2.1 Atlantic redfish (Sebastes marinus)

The Atlantic redfish (*Sebastes marinus*) (Figure 1) is bright red or orange-red in colour. The maximum average size is 55-70 centimeters, but mostly industrial fisheries catch fish around 30-45 centimeters weighing between 0.6 to 2 kg. Fillets of redfish are firm and white in colour. Redfish is a pelagic fish found at depths between 300 and 1,000 meters, but inhabits deep continental shelves mainly at depths between 100 m and 300 m. It feeds on a variety of food organisms including a diversity of small fish (Saborido-Rey, Garabana, Stansky, Melnikov, & Shibanov, 2005). In contrast to most fish that spawn unfertilised eggs, redfish has internal fertilisation and spawns free-living larvae. Females are larger than males. Atlantic redfish are caught by bottom long line and bottom trawls. The most important fishing areas for redfish are in the Northwest and Northeast Atlantic (Stransky, 2005).

The content composition of 100 grams of fresh Atlantic redfish include 18,7 g protein, 3.9 g fat, 3,645 mg Omega fatty acid and 84 mg Sodium (Fisheries, 2017). The fish can be utilised as fresh, frozen, eaten fried, boiled, microwaved and baked as whole fish or fillets.



Figure 1. Atlantic Redfish (Sebastes marinus)

2.2 Atlantic herring (Clupea harengus)

The Atlantic herring (*Clupea harengus*) (Figure 2) is a small oily fish with a dark blue green or steel blue upper part of the body, blackish blue snout and silvery sides and belly. The average length is 23-36 cm but occasionally it can reach up to 43 cm. Atlantic herring is a pelagic species found at depths down to 400 m, and feeds mainly on zooplankton, particularly the tiny copepod called *Calanus*, near the surface. Atlantic herring has external fertilisation, laying 20,000-40,000 eggs which are sticky and attach themselves to the sea bed usually in waters 10-80 m deep, on hard ground covered with small stones, shells or seaweed (Stroud, 2001).

Herring is found on the north Atlantic, the Baltic Sea, North Pacific, and the Mediterranean. The most important fishing grounds are the North Sea, the Baltic Sea, and the coastal waters of Britain, Norway, Iceland and Canada. There are 15 species of herring, but the most abundant is the Atlantic herring (*Clupea harengus*). They move in vast schools, appearing in spring at the shores of Europe and America, where they are caught, salted and smoked in great quantities (Stroud, 2001).

The chemical composition of Atlantic herring varies considerably with the season and life history stage. The fat, protein and water contents of whole herring may be less than 1%, 17% and 60% respectively (right after spawning), or more than 20%, 21% and 80% respectively, before spawning season (Burt & Hardy, 1992). Herring is rich in minerals such as iron, calcium, iodine and vitamins (Stroud, 2001). Shelf life in ice for high fat (summer herring) and low fat (winter herring) fish is 2-6 and 7-12 days, respectively (Huss, 1995).



Figure 2. Atlantic Herring (Clupea harengus)

2.3 Shelf life of fish

The shelf life of fish is defined as the time from when the fish is caught until it is no longer fit for consumption (Huss, 1995). Some experts define shelf life as the maximum period during which the pre-determined quality attributes of foods are retained (Daun, 1993).

Freshness as a quality aspect is important because it explains bio-chemical, microbial, and physical changes in fish over time from when the fish is caught (Olafsdottir, et al., 1997). Sensory changes in fish freshness can be used to determine the shelf life of fish, by evaluation of appearance of the skin, eyes, gills, firmness, elasticity, and smell or odour (Olafsdottir, et al., 1997). Sensory characteristics of fish change depending on species and storage methods. Fish spoilage can be divided into four distinct phases. Phase 1: Very fresh, sweet, marine sea weed. Phase 2: Loss of characteristic odour and taste, flesh neutral (no off-flavours). Phase 3: There is a sign of spoilage and a range of volatile trimethylamine (TMA) derived from the bacterial reduction of trimethylamine oxide (TMAO), causing an unpleasant smell or odours/flavours. It starts with slightly sour, fruity flavours and late stage smell develops to

cabbage like, ammonia, sulphurous and rancid, the texture becomes soft, watery or dry and tough. Phase 4: The fish is spoiled, putrid and rotten.

Changes in phases 1 and 2 are mainly due to autolytic reactions, but in phases 3 and 4 the major changes are due to bacterial activity (Huss, 1995), (Church, 1998). Therefore, a shelf life study should mainly be conducted by sensory methods together with microbiological, chemical and physical measurements.

Several factors influence the shelf life of fish. Handling is critical, especially quick cooling after catching. Bleeding is also important, along with fishing gear, temperature fluctuation in storage, seasonality and fishing grounds (Martinsdottir, Sveinsdottir, Luten, Schelvis-Smith, & Hyldig, 2001). Fish size is one of the most important factors that influence shelf life. Large, round and fatty fish require more initial ice for sufficient time for inner layer to be chilled (Huss, 1995). Initial storage temperature can influence shelf life of fish and fish products by showing enzymatic activity, and growth of aerobic specific spoilage organisms (SSO). Cooling together with use of modified atmosphere packaging (MAP) generally results in increased shelf life as compared with traditional ice storage (Magnússon, Sveinsdóttir, & Porvaldsson, 2010).

Rigor mortis can also indicate quality, freshness and shelf life of the fish (Rodríguez-Jérez, Hernández-Herrero, & Roig-Sagués, 2004). When rigor mortis occurs after the fish is killed, it forms actin and myosin linkages due to lack of muscle ATP, then dissolution of rigor mortis, autolysis, and finally to bacterial spoilage. The onset and strength of rigor mortis affects fillet quality. Fasts autolysis increases ruptures of muscle tissues, resulting in increased gaping, flesh softening and reduced shelf-life of the fillets. Onset, length and resolution of rigor mortis are influenced by temperature, time, handling, size and physical condition of the fish (Huss, 1995). Therefore, proper chilling of the fish when it is caught extends the shelf life.

2.4 Sensory evaluation of fish

Sensory evaluation can be defined as a scientific way used to measure, analyse and interpret characteristics by sense of sight, smell and touch (Stone & Sidel, 2004). The sensory characteristics of fish is mainly for consumer satisfaction; hence it offers immediate quality information. Sensory evaluation is a rapid and formal way to assess fish freshness in trade, however it is not always objective and documented (Alasalvar, et al., 2001).

2.4.1 Quality Index Method (QIM) Scheme

QIM is an ideal method for training of individuals such as fishers regarding the quality of their catch and influences better handling of fish on board (Hyldig, 2004). QIM is based on a scheme developed by the Tasmanian Food Research Division (CSIRO) and has demonstrated rapid and satisfactory estimations for the freshness and quality of seafood for many fish species.

QIM involves the selection of appropriate and best fitting attributes to observe a linear increase in the Quality Index (QI) with storage time in ice or at different temperatures (Martinsdottir, Sveinsdottir, Luten, Schelvis-Smith, & Hyldig, 2001). It may be used to determine the remaining shelf life and maximum storage time for fish and fish products at different temperatures. The maximum storage time of fish until when it is unfit for human consumption can also be determined by the sensory evaluation of cooked samples using Generic Descriptive Analysis (GDA). The development of QIM for fresh fish may use GDA results as a reference (Sveinsdottir, Hyldig, Martinsdottir, Jorgensen, & Kristberrgasson, 2003).

In developing QIM, experienced panelists develop best descriptors for precise quality parameters that can be used to predict changes and remaining shelf life of the spoiling fish (Sveinsdottir, Hyldig, Martinsdottir, Jorgensen, & Kristberrgasson, 2003). Then the drafted

QIM is used by the panel in several sessions to observe changes. Feedback and reintroduction of the developed QIM must be done under panel leader supervision. The developed QIM scheme is finalised by a shelf life study of the fish (Hyldig, Bremer, Martinsdottir, & Schelvis, 2007). The scheme uses score from 0 to 3 demerit (index) points which is given for each quality parameter according to the specific parameter descriptions. Each description of the individual grades is precise, objective, independent and primary rather than a cluster of terms. Scores are summarised to give an overall sensory score called the Quality Index (QI) resulting in estimates of the past and remaining shelf life when it gives linear results related to the storage time (Bonilla, Sveinsdottir, & Martinsdottir, 2007) (Sveinsdottir, Hyldig, Martinsdottir, Jorgensen, & Kristberrgasson, 2003). For example, a developed QIM scheme of fresh cod fillets showed a clear linear relationship to storage time on ice and maximum shelf life of 8 days based on counts of H₂S-producing bacteria and sensory evaluation with Quantitative Descriptive Analysis (QDA) (Bonilla & Sveinsdottir, 2005).

QIM is used as a quality assessment tool for fish products and was initially developed for whole fish but has been further developed for raw fish fillets, for example, fresh cod fillets (Bonilla, Sveinsdottir, & Martinsdottir, 2007) and haddock fillets, (Olafsdottir, Lauzon, Martinsdottir, & Kristbergsson, 2006). Sensory changes of the fillets as descriptors for colour, odour, texture, gaping and appearance, or any other important parameter, are specific to different fish species. Together with Generic Descriptive Analysis (GDA) method, QIM can be used to determine the storage time for cooked fish fillets and gives a detailed description of fish fillet sensory profile when the fillet is no longer fit for human consumption (Bonilla, Sveinsdottir, & Martinsdottir, 2007).

Different cooling techniques have a different influence on sensory attributes. Therefore, QIM needs to be adapted for quality assessment considering different storage conditions and characteristics of various fish species. QIM for various species have been developed and made easily accessible online at website (<u>http://www.qim-eurofish.com/</u>).

2.4.2 Descriptive analysis

Descriptive analysis is a methodology that provides quantitative description of products, based on perceptions from a group of qualified panellists. A commonly used descriptive test is Quantitative Descriptive Analysis (QDA), a sensory method in which maximum storage time or shelf life fish and detailed description of the sensory profile for a product is determined by sensory evaluation of cooked samples (Stone & Sidel, 2004). The method was developed by Tragon Corporation under partial collaboration with the Department of Food Science at the University of California, Davis. The intention was to respond to dissatisfaction among sensory analysts with lack of statistical treatment of data obtained with sensory profile of flavour or related descriptive methods (Meilgaard, 1991).

Descriptive analytical methods have different approaches, but the basic framework of all the techniques are the same. However, among all descriptive methods only Generic Descriptive Analysis (GDA), which is to be used in this project, generally takes elements from QDA and spectrum methods that support the interest of product developers. It uses panellists to fully describe attributes and rates intensity differences from reference control point over time, and allows product profile to be compared across each product at the same time by minimising panellist drift (Meilgaard, Civille, & Carr, 2007). GDA allows for modifications to meet specific project objectives and limitations of the product being tested during practical applications (Lawless, 1998), (Murray, 2001). A basic strength of the GDA method is the ability to independently verify (after each test) that panellists perceive differences among products on attributes in a reliable manner.

The training for GDA requires the use of product and ingredient references to stimulate consistent terminologies to be used during evaluation. The terminologies are first developed during an interactive session with guidance from the panel leader, but the panel leader refrains from influencing the group. Panellists are then familiarised with the attributes and develop definitions on using the unstructured scale. The panellists are free to develop their own approaches to scoring using unstructured 15 cm (6 inches) line scale (0-100%) which indicate relative intensity of the attribute by making a mark on the line. The scale direction is always from left to right with increasing intensity. Then panellists participate in the evaluation process that is carried out in separate booths to reduce distraction and panellist interaction (Meilgaard, 1991). The evaluation is highly dependent on the commitment and motivation of the panellists. Panel performance can be examined by interaction of product and panelist. The QDA results of attributes in the same sensory category are statistically analysed and can be graphically presented by a "spider web" from the central point of each attribute (Lawless, 1998), (Meilgaard, 1991).

GDA is a useful additional sensory method to QIM for determining shelf life of fish based on the perceived sight, odour and flavour of the product (Sveinsdottir, Hyldig, Martinsdottir, Jorgensen, & Kristbergsson, 2002). Determining the maximum shelf life of a product using GDA is done when the panel or part of the panel detects spoilage attributes in the samples, or when the samples are increasingly described with words applying to spoilage attributes. QDA as a reference to enable prediction of the remaining storage time of raw salmon in ice was found to be 20–21 days (Sveinsdottir, Hyldig, Martinsdottir, Jorgensen, & Kristberrgasson, 2003). Other applications include its use in measuring shelf life of products without dependence on standards or control products. Also, GDA is used for comparison of acceptability of products stored in different condition such as fresh or frozen for short and extended time storage. Few studies for comparing consumer acceptability and sensory properties have been conducted (Sveinsdóttir, et al., 2009).

Applications of GDA vary and the methodology has been used for the resolution of complex data from both chemical and physical analysed. It has been used to organise information on which quality characteristics are considered the most important to consumers (Stone & Sidel, 2004). Seafood buyers and consumers use taste evaluation to ensure that the product meets their expectation and acceptability limits (Botta, 1995).

2.5 Microbial analysis

Microbial analysis can be used to estimate shelf life of fish products by periodically measuring the number of specific spoilage bacteria which is related to the remaining shelf life (Huss, 1995). The amount of microbial load in fish habitat can influence counts of bacteria that contribute to short shelf life of the product, as a considerable number of bacteria are present in skin, gills and viscera (Oehlenschlager & Sorensen, 1997). The number of bacteria on newly caught fish can vary greatly, normally ranging from 10^2 to 10^7 cfu/g (Liston, 1980). Microbial metabolites have low odour thresholds and the number of bacteria increases rapidly in the flesh. During spoilage, the concentrations of sulfur compounds, short-chain acids, alcohols and amines increase (Olafsdottir, 1997). Bacteria use various compounds to grow, resulting in increasingly bad-smelling sulphur and nitrogenous volatiles until most people evaluate the fish unfit for consumption (Martinsdottir, Sveinsdottir, Luten, Schelvis-Smith, & Hyldig, 2001). Seafood spoilage bacteria are characterised by their ability to produce H₂S and reduce trimethylamine oxide (TMAO), which has been used for their specific determination.

Total Viable Count (TVC) using pour plate method with different peptone-rich substrates containing ferric citrate have been used for H_2S -producing bacteria detection, such as

Shewanella putrefaciens, in which, due to precipitation of FeS are recognised as black colonies (Huss, 1995). Specific spoilage bacteria to cause spoilage in iced fish when stored aerobically levels of 10^8 - 10^9 cfu/g in the flesh are required (Gram & Huss, 1996).

3 MATERIAL AND METHODS

3.1 Experimental design

3.1.1 Proposed QIM scheme for thawed redfish fillets

The methodology used in this study was guided from previous works on development and application of QIM scheme for fresh cod (*Gadus morhua*) fillet and application in shelf life study (Bonilla, Sveinsdottir, & Martinsdottir, 2007).

Raw fillets of wild red fish without skin were bought from HB Grandi company. The red fish was caught by trawler, stored whole in ice after catch and filleted after 3 days for the first batch and 5 days for the second batch. At the laboratory, the first batch of fillets for pre-observation was received on 11/12/2017 and second batch on 03/01/2018 stored at -25°C in boxes of 8-10kg insulated within one plastic layer before being packed in the box. Samples were thawed (spreading form) and stored at 0-4°C until randomly sampled for analysis with QIM, GDA and microbial counts.

The study procedures were divided into three stages (Figure 3). In the initial stage, two fillets for each storage day were pre-observed for sensory changes and the results were used for the formulation of a preliminary scheme. Then, three fillets for each storage day were used in all QIM training sessions, ten fillets for each storage day GDA training and improvement of the scheme. The final stage was completed by evaluation using the developed scheme and one fillet for each storage day was randomly sampled for microbial analysis to determine shelf life.



Figure 3 Experimental design for the development and application of QIM scheme for Red fish (*Sebastes marinus*) fillets stored at 0-4°C.

Temperature loggers

A

I Button temperature logger (DS1922L/T) was used to measure and record temperature every 10 minutes and was placed in the storage freezer (-25 0 C) and a second logger (Onset UTBI-001 Tidbit v2 temperature logger) was placed in cold room (0-4 0 C) about 50 cm from the floor close to the spread fillets and recorded temperature every 10 minutes.



Figure 4. Temperature loggers A (I Button temperature logger (DS1922L/T) and B (Onset UTBI-001 Tidbit v2 temperature logger) installed at -25° C and $0-4^{\circ}$ C

3.1.2 Proposed preliminary scheme for thawed whole herring (Clupea harengus)

Whole thawed herring was used to develop a pre-observation stage of QIM only (Figure 5). The herring was caught in the North Atlantic by using midwater trawler/ purse seiner and stored at temperature of -25 ⁰C from production day October 08, 2017 in thick plastic bags of about 12 kg. This is the recommended fishing season due to stable fat content for processing after the spawning (April -June).

Initial thawing was done at room temperature for 12 hours overnight. Then 21 samples were further thawed (spreading form) in a cold room with temperature 0-4°C ready for experiment. Three samples were collected randomly on 1, 3, 6, 9, 12, 15 and 18 days and observed for changes over time using drafted QIM for thawed whole herring. On the last day of pre-observation, a preliminary QIM scheme for whole thawed herring was completed.



Figure 5. Experimental design for QIM application to pre-observation of thawed whole herring stored at 0-4 $^{\circ}$ C for up to 18 days.

3.2 Sensory evaluation of thawed red fish fillets using Quality Index Method (QIM)

The procedure for preparation was conducted under standardised conditions: samples placed on clean white sheets, samples left for about 15-30 minutes and covered with plastic sheets to prevent dryness at room temperature. The samples were displayed to evaluate attributes on the

back-bone side and skin side of fillets for each storage time. Samples were coded, by random written numbers on yellow labels placed next to each fillet with random numbers not indicating the storage time.



Figure 6. Fillets prepared for QIM evaluation displaying back bone side(left) and skin side (right).

3.2.1 Development of QIM scheme for thawed red fish fillets

Pre-observation of thawed raw fillets was carried out to observe the changes occurring in quality parameters with storage time at 0-4°C after thawing. Two persons observed three fish fillets after 1, 2, 5, 7, 8, 10, 13 and 16 days of storage at 0-4°C. The changes occurring in gaping, texture, color, odour, and appearance were listed in a grading scheme, that used Githu's QIM scheme descriptions for deskinned fresh red fish fillets as reference using whole numbers ranging from 0, for fresh, to 3 pending on freshness decline (Githu, 2013). Based on these observations, some sensory parameters were modified and attributes on the back-bone side and skin side were recorded and analysed. Based on the results acquired, a preliminary QIM scheme for the sensory evaluation of thawed fillets was established.

The preliminary QIM scheme was used to evaluate the quality parameters of the thawed red fish fillets in all sessions. Ten trained sensory panellists participated in two training sessions and were familiarised with conducting QIM evaluation of red fish fillets. Three fillets from six different storage days (1, 2, 6, 7, 11 and 13) were evaluated. Storage time was shown in both first and second sessions.

During training sessions, the panel leader explained how to evaluate each quality parameter using the QIM scheme which was developed during the pre-observation. Then the panel proceeded to evaluate the thawed fillets and gave feedback during the process. After each training session, the panellists discussed the scheme and gave suggestions for improvement of the scheme. Some changes were made in the scheme during the training sessions. During the final training session, the panellists were informed of all relevant changes made to the scheme based on their prior discussions and feedback, the QIM scheme for thawed red fish fillet was completed.

3.2.2 Evaluation of freshness using QIM scheme in a shelf life study

The QIM scheme developed was used to evaluate the thawed fish fillets in two sessions by the sensory panel. The panellist evaluated 3 coded fillets with random 3-digit numbers without information about the storage time from the 5 different storage days (1, 4, 7, 9 and 12).

3.3 Sensory evaluation of cooked fillets using GDA

Samples weighing about 40–50 g were taken from the loin part of the fillets, divided into two pieces tail and head parts and placed in aluminium boxes coded with three-digit random numbers. The samples were cooked at 95–100 °C for 6 min in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfng, Germany) with air circulation and steam.



Figure 7. Preparation of cooked samples for GDA

3.3.1 Training on GDA

Ten trained sensory panellists experienced in sensory evaluation of thawed redfish fillets were trained during two QIM training sessions and were familiarised with list of sensory attributes of cooked redfish fillets in one training session. They were given a list of words to describe appearance, odour, texture and flavour of the cooked fillet samples, and were trained to describe the intensity of each attribute for a given sample using an unstructured scale, from 0 to 100%.

The training on GDA of cooked redfish fillets was conducted in parallel to the second QIM training session. Each panellist evaluated 2 samples (A and B) in the sensory booths. The intention was to obtain a primary precise independent description of the cooked fillets before the whole panel sat for evaluation and discussion of the samples. Then, 3 samples (A, B and C) of cooked redfish fillets, which made a total of five samples in one session (1, 6 days sample in sub-session 1 and 1, 6, 11 days samples in sub-session 2), whereby coded letters used A, B and C represented the three storage times (1, 6, 11 days). The panellists described the intensity of the attributes utilised during training and a suggested improvement of the scheme. The GDA scheme was then completed for shelf life study of the cooked fillets.

3.3.2 Evaluation of shelf life using GDA

Nine panellists evaluated the cooked samples that were coded with three-digit numbers without information about the storage time by using a list developed during training as by Stone & Sidel, (2004). This time the panellists were already trained, but had one refresher training session. Each panellist evaluated randomly in duplicate 3 samples from six different storage times. A computerised system (FIZZ, Version 2.50b, 1994–2015, Biosystémes) was used for data recording and for further processing. The scores of panellists were calculated for each sample assessed and the reported value was the average of the duplicate samples.

3.3.3 Microbial counts

Flesh samples for microbial analysis were collected from fillets of 1, 4, 9 and 14 days of storage before commencing sensory evaluation. Samples of 20 g from each fillet were minced separately with a blender and placed in a stomacher bag containing 180 g of Maximum

Recovery Diluent (MRD, Oxoidi, UK). The homogenization in a stomacher bag was done for one minute and successive serials of 10-fold dilution were done as required. After dilution 1ml of 1/10 (10-fold) solution was transferred to a petri dish and overlaid with a solution of iron agar (IA) by the pour plate technique.

The plates were incubated at 17 °C for 5 days. The bacteria were identified as both black and white colonies on this medium. Total viable psychrotrophic count (TVC) was done by counting number of colonies (both black and white) using colony counter and the to count H₂S-producing bacteria by count of black colonies in CFU/g. On days 1, 7 and 12 the same sample amount and procedures were done but, spread plate method was performed on Plate Count Agar (PCA) and the results obtained were total viable psychrotrophic counts (TVC).

3.4 Pre-observation of sensory changes in whole thawed herring

The preparation procedures were conducted under standardised conditions which involved putting samples on a clean white sheet, for about 15-30 minutes covered with plastic sheet to prevent dryness at room temperature. For each storage time, three samples were randomly taken for pre-observation on days 0, 3, 6, 9, 12, 15 and 18. Observation was done on whole thawed herring to evaluate the whole fish (appearance, texture of loin, texture of the belly and odour), eyes (appearance of the pupil, shape), gills (colour/appearance, mucus odour) and then carefully dissected for observation of the abdomen (blood colour in abdomen, odour, appearance of gut content). The changes occurring in parameters were listed according to a grading scheme in whole numbers ranging from 0, for fresh, to 3 pending on spoilage increase, reference and modifications adapted from frozen whole herring (Nielsen & Hyldig, 2004). During these observations, some sensory parameters were modified from initial drafted QIM for thawed whole herring and attribute changes were recorded. Based on the results acquired, a preliminary QIM scheme for sensory evaluation of whole thawed herring was established which can be used for further development of QIM scheme for thawed whole herring in the future.

4 RESULTS

4.1 Temperature

Two temperature loggers were placed in redfish fillets, one logger was placed inside the box of fillets and one outside kept in frozen storage (Figure 8a) during the life span of the experiment starting from 14/12/2017 to 12/01/2018. One temperature logger was placed in the redfish storage room used for thawing the fillets and whole herring thawing in thawing storage room (Figure 8b).

Average temperature during frozen storage inside the fillets ranged from -24.1 ± 2.5 °C with a slight fluctuation on the first day when the logger was placed and the outside temperature fluctuation was mostly due to opening of the freezer. During the last days of the experiment, temperatures increased slightly both inside and outside the redfish fillet boxes due to malfunction of the freezer fan. Highest inside temperature of the fillet was -15.9 °C which was still safe for the fillets (Figure 8a). Average temperature in the room used to thaw the fish was 1.7 ± 1.5 °C with high increase on day 15 where the logger was retrieved for hours as to prepare the place for next setup of thawing herring at same storage (Figure 8 b).

Mlingi





Figure 8. a) Temperature log of frozen storage of redfish fillets outside and inside the box at - $25 \,{}^{0}$ C and b) thawing storage room temperature (1.7±1.5 °C).

4.2 Development of QIM scheme for thawed redfish fillet

The QIM scheme development was aimed at identifying visible parameters that changed noticeably on both the backbone and skin side of skinned fillet for gaping, colour, texture, odour, appearance and dark muscle colour which were noted as the main observable parameters that changed clearly with storage time. The final developed scheme showed increase in Quality index score (QI) with increase in storage time with maximum QI score of 22 points (Table 1).

In the preliminary scheme, two parameters, backbone side and skin side, with six attributes were listed during pre-observations of thawed red fish fillets (Appendix 1). The maximum sum of the quality index points was 18.

During the training sessions, modifications were made to the description of some attributes. More suitable descriptive words were used to describe attributes of colour, odour, gaping and attributes for appearance of both backbone and skin side was introduced. The scheme was finalised after the last session of training which contained two parameters for backbone and skin side with eight attributes and the total sum of quality index points was 22 (Table 1).

	Description		Score						
Backbone	Gaping	No gaping, one split in the thick loin or neck part of the fillet	0						
side		Slight gaping, flesh torn less than 25 %	1						
	More gaping, flesh torn 25-50 %		2						
	Deep gaping over 50%, flesh more torn								
	Colour	Reddish, bluish greyish (small clear spots on dorsal edge of the fillet).	0						
		Slightly milky (small light brown spots on dorsal edge of the fillet)	1						
		Milky, slightly brownish, slightly yellowish at the edges							
		Brownish, yellowish, greenish at the edges part	3						
		No mucus, matt							
	Appearance	Shiny mucus film on the edges of the fillet	1						
		Shiny mucus film more than 25% of the fillet	2						
	Texture	Firm	0						
		Rather soft /fairly firm	1						
		Soft	2						
		Very soft	3						
		Fresh, marine	0						
	Odour	Cold room, starchy, cucumber, melon	1						
	Odoui	Sour, rancid, over ripened fruit, dried fish	2						
		Very sour, putrid, TMA, rotten	3						
Skin side	Dark	Bright, red brown, slightly bluish	0						
	muscles	More brown, patchy reddish in some areas	1						
		Brown-greyish, slightly yellow at the edges	2						
		Brownish, yellowish-greenish at the edges	3						
		Bright iridescent no-mucus	0						
	Appearance	Shiny mucus film near edges	1						
		Shiny mucus film on most of the fillet	2						
	Odour	Fresh, marine, cod liver oil	0						
		Cold room, starchy, melon, cucumber	1						
		Sour, rancid, over ripened fruits							
		Very sour, putrid, rotten	3						
	Quality index	x (22)							

Table 1. Quality Index Method scheme developed for thawed redfish (*Sebastes marinus*) fillets

4.3 Shelf life study

4.3.1 Evaluation of raw fillets using QIM

Thawed redfish fillets stored at 1.7 ± 1.5 °C were evaluated. After 1 day of storage the fillets were matt and bright irridescent with no mucus, reddish bluish greyish on the backbone side and while muscle colour was bright or red-brown on the skin side (9 a, c). On the last days of evaluation, a shiny mucus film covered more than 25% of the fillet which was milky, brownish and yellowish at the edges, dark muscles colour brown-greyish, yellow at the edges respectively (Figure 9 b, c). Odour was detected as having a cucumber, melon, starchy, cold

room smell that progressed to over-ripe fruits, sour, rancid, dried fish, while the texture changed from firm to soft with finger marks disappearing quickly within 3 seconds and gaping was less than 25% throughout storage time.

Figure 9. Fillets with different storage time

9a: Fillets after 1 day storage time showing both sides

9b: Fillets after 14 days of storage showing both sides

9c: Fillets after 1, 7 and 12 days of storage showing both sides (3 fillets for each storage time beginning from left).

The QI scores for each quality parameter showed a moderate linear increase with storage time. The lowest score and highest score for 1 and 12 days of storage respectively (Figure 10). The average scores for all evaluated parameters were around 0-1 for fillets on day 1 of storage. All attributes for backbone parameters received lower scores after 9 than after 7 days of storage. The average score of fillets after 12 days received highest score for odour (both backbone and skin sides), dark muscle and colour. The scores for gaping and texture did not increase with storage time. The scores for gaping and texture were similar on day 9 and day 1.



Storage days



Figure 10. Average score for each quality attribute evaluated with QIM scheme for thawed redfish (Sebastes marinus) fillets.

The QI based on the average of the whole panel (9-10 panellists) was calculated for each trial day of storage. The QI was linearly related to storage time (Figure 11) with the correlation R^2 =0.7275. However, when gaping and texture attributes are removed due failure to show linear increase with storage days which can be due to fillets handling, the correlation is improved to R^2 =0.8156. The average score for fillets after 7 and 9 days were slightly different from fillets after 4 days of storage by average QI 0.37 and 0.02 respectively whereby fillets after 9 days of storage showed a lower average QI score than fillets after 4 days of storage.



Figure 11. Quality Index of redfish fillets over storage days 11a (with all attributes), 11b (without gaping and texture attributes).

Each fillet at each storage time generally showed a similar trend with slight variation in its average score with the exception of fillets after 9 days of storage which showed the variation between the 3 fillets where 2 fillets ranked even lower than the 3 fillets after 7 days of storage (Figure 12 and table 2 below). At the end of the storage time, each fillet score reached values closer to the maximum score (0-22) which is also shown by high standard deviation in table 2.

Stanaga dava	Average QI score /Standard deviation							
Storage days	Fillet 1	Fillet 2	Fillet 3					
1a	6.44±1.96	4.72±2.03	5.56±2.84					
1b	6.50±2.36	6.15±2.19	6.00±2.38					
4	6.75±2.83	7.60±1.76	9.25±1.75					
7	8.78±2.68	8.28±1.35	7.67±2.37					
9	10.50±2.67	6.75±2.28	6.30±2.25					
12	14.33±3.43	13.67±3.57	12.75±3.09					

Table 2. Distribution of quality index (QI) mean value and standard deviation of each red fish fillet with each storage day.



Figure 12. Distribution of quality index score of each red fish fillet with each storage day. (Numbers and letters differentiate 3 fillets that were evaluated for each storage day, but fillets with 1 day of storage were evaluated twice in different days).

In the two sessions for shelf life study 9-10 panellist evaluated, but one assessor (assessor 3) was not present on the first day. A total of 10 panellists participated, and everyone took part in the final training session. The individual panellists participating in the QIM evaluation of redfish fillets performed differently, as there was slight variation in the QI score obtained (Figure 13). Panellists average scores showed disparity between panellists on the first days of storage. The lowest score from the whole panel was on day 1, highest score was on storage day 12, which indicated that the panellists agreed more when evaluating the samples at first and last days of storage. However, panellist 6 scored very high at storage day 1, panellist 9 scored very high and panellist 4 scored very low on the last storage day 12 which indicated more training is required.

Storage day 9 was scored lower than day 7 and day 4 by 60% of the panellists (1, 2, 4, 6, 7 and 10), where panellist 10 scored less for day 9 than storage day 1. The scores of sensory evaluation panellist numbers 5 and 8 performed best throughout and panellist 3 scored within the range in spite of absence in one evaluation session.



Figure 13. Quality index score of different panellists with storage time.

4.3.2 Evaluation of cooked fillets using GDA

On generic descriptive analysis (GDA) sensory panel evaluated odour, flavour, appearance and texture (Table 3).

Sensory att	ributes	Description of attribute			
Odour	sweet (+)	sweet odour			
	cod liver (+)	boiled cod liver, metallic odour			
	shellfish (+)	characteristic fresh odour			
	dish cloth (-)	dirty damp kitchen cloth			
	rancid (-)	rancid odour			
	TMA (-)	TMA, dried fish, amine			
	overripe fruit (-)	spoilage characteristic, overripe fruit, queasy sweet			
	sour (-)	spoilage sour, sour milk, vinegar, butyric acid			
	sulphur (-)	sulphur, matchstick, boiled cabbage, turnips			
spoilage (-)		spoilage odour (rancid, sour, TMA, overripe fruit, sulphur)			
Appearance	colour	sample surface. light; white colour. Dark; yellow, brown,			
	heterogeneous	sample surface. Heterogeneous colour; e.g. Spots, discolou			
Flavour	metallic (+)	characteristic metallic flavour of fresh, white, lean fish			
	fish oil (+)	fresh fish oil, fresh liver (not rancid)			
	sweet (+)	characteristic sweet flavour of fresh boiled redfish			
	bitter (-)	bitter flavour			
	overripe fruit (-)	spoilage characteristic, overripe fruit, queasy sweet			
	sour (-)	spoilage sour			
	TMA (-)	TMA, dried fish, amine			
	rancid (-)	rancid flavour			
	putrid (-)	putrid			
	spoilage (-)	spoilage flavour (rancid, sour, TMA, overripe fruit, sulphu			
Texture	juicy	when chewing, juicy: releases liquid, dry: draws liquid			

Table 3. Sensory Attributes of cooked red fish fillets samples assessed by GDA method. Freshness attributes are marked with (+), spoilage indicating attributes with (-)

Fillets after 12 days of storage were observed to have high spoilage characteristics during QIM evaluation and were not evaluated using GDA.

At the beginning of storage, freshness attributes describing the odour and flavour during the first 4 storage days were dominant and decreased towards end of storage time. Most attributes indicating spoilage were slightly detected and beginning to increase from day 4 to the final day

of storage. However, freshness attributes of shellfish-odour and fish oil, metallic-flavours slightly decreased on fillet of 1 day and second day evaluated (1b) on second day of storage, and negative odour attributes of dish cloth and sulphur decreased from day 7 to last days of storage (Table 4).

Storage days	1-1		1-2		4		7		9		p-value
Sensory attributes											
Odour											
sweet	27		26		23		18		17		0.029
cod liver	14		17	a	14		10		9	b	0.030
shellfish	14	a	10		13	a	8	b	7	b	0.006
dish cloth	3		1		2		10		8		0.044
rancid	1		2		3		4		5		0.123
TMA	1	b	2		3		3		8	a	0.053
overripe fruit	0	b	1	b	3	b	4	b	10	a	0.002
sour	1		0		2		3		3		0.325
sulphur	1		0		1		2		1		0.333
spoilage	2	b	2	b	6		9		14	a	0.008
Appearance											
colour	23		25		26		25		25		0.982
heterogeneous	22		32		32		26		23		0.084
Flavour											
metallic	22	a	13		17		17		9	b	0.007
fish oil	24	a	20		25	a	19	a	14	b	0.001
sweet	16	a	16	a	14	a	10		4	b	0.009
bitter	7	b	6	b	8	b	9	b	17	a	0.003
overripe fruit	3	b	6	b	4	b	4	b	12	a	0.001
sour	2		4		3		3		8		0.453
TMA	3	с	3	с	6	bc	12	ab	21	a	0.000
rancid	1	b	4	b	2	b	9		13	a	0.002
putrid	0	b	4	b	3	b	4	b	16	a	0.012
spoilage	5	b	8	b	4	b	11	b	32	a	0.000
Texture											
juicy	54		56		50		53		53		0.526

Table 4. Mean sensory scores for attributes of cooked red fish fillets. Different letters (a, b, c) indicate significant different values between samples within a line.

Odour characteristic for cod-liver and shellfish attributes, were detected as moderate when compared with sweet attributes which were highly detected on 1 day of storage and together these attributes decreased with storage (Figure 14a and Table 4). Odour characteristics dishcloth and sulphur decreased after day 7 of storage, while TMA, spoilage and overripe fruit, attributes increase rapidly. Spoilage attributes were detected slightly on fillets at 1 day storage and increased rapidly from day 4 to the end of storage. The attributes for rancid and sour showed a gradual increase after storage day 4 compared to other spoilage odour attributes (Figure 14b and Table 4).



Figure 14. Freshness (a) and spoilage indicating (b) odour attributes score of cooked red fish fillets against storage days.

The freshness flavour characteristics metallic, fish oil and sweet attributes decreased strongly with storage days especially after day 4 and slightly fluctuated with fillet of day 1 second day evaluated (1b) for metallic and fish oil, while sweet received same score for both fillets of 1 day (Figure 15a). The spoilage flavour attributes bitter, rancid, putrid, overripe, TMA increased with storage days, but spoilage attribute score very high on last day of storage and sour detection was low throughout the storage days (Figure 15b).



Figure 15. Freshness (a) and spoilage indicating (b) flavour attributes score of cooked red fish fillets against storage days.

The appearance attributes showed that the fillets had constant colour throughout the storage days, but were slightly lighter on first fillet of day 1. Also, the fillets had heterogenous appearance that highly increased on second fillets of day 1 and 4 days of storage but showed a decreasing trend towards last days of storage (Figure 16a and Table 4). Texture attributes showed high juicy released when fillets of 1 day of storage were chewed and very slight decrease on fillets with 4 days of storage that showed less juicy but stabilised with increase of storage days (Figure 16 b and Table 4).



Figure 16. Appearance (a) and Texture (b) attributes score of cooked red fish fillets against storage days.

4.3.3 Microbial counts

The microbial counts showed a linear increasing trend with storage time (Figure 17). At the beginning of storage on day 1 the TVC was around $1.3*10^4$ cfu/g and $2.7*10^4$ cfu/g. H2S-producing bacteria was $2.0*10^2$ cfu/g. based on sampling days 1b, 4, 9 and 14. At the end of storage, on day 14 an increase was observed reaching to $5.4*10^8$ cfu/g for TVC and $1.3*10^8$ cfu/g for H₂S-producing bacteria.



Figure 17. Total viable counts and counts of H2S-producing bacteria in red fish fillets against storage days.

4.4 Pre-observation of sensory changes in whole thawed herring

The samples of whole thawed herring stored at 1.7 ± 1.5 °C were observed for the sensory changes, involving quality parameters of whole fish, eyes, gills and abdomen content (Figure 18).

On day 1 the body had a less slivery skin, eyes were flat with slight opaque pupil, red gills but not bright-red, gut content was fresh with some red coloured blood and organs were in good shape (Figure 18). During the last days of pre-observation, from 15 to 18 days of storage, the

whole fish colour was dull, slight yellow at the lower part of abdomen with small mould spores on the skin especially the head part, sunken/concave eyes, and gills became pinkish-brown.

Abdominal contents were destroyed and turned brownish, especially delicate organs and edges of gonads and egg pockets; blood was dark brown-blackish with presence of some brownish thick fluid. The body odour changed from slight fresh sea-neutral on the first days to fermentation remains, yeasty and with a strong off odour and gill odour which changed from grassy to sour grassy, yeasty fermentation remained, and finally a strong rotten smell. The gut content odour changed from neutral cucumber, kitchen cloth, sour with a strong rotten cabbage odour on the last day of storage.

During the pre-observation the clearly visible changes with storage time was easilu seen by abdominal content and texture of the inner flesh when dissecting the abdomen among all quality parameters used in the study. Observation of sensory changes after being in 18 days of storage was used to formulate of preliminary QIM scheme (Appendix 2).



Mlingi





18 Days



Figure 18. Sensory changes in appearance of thawed herring from storage days 1,12 to 18.

5 DISCUSSION

5.1 Development of the scheme

Visible deteriorative changes occurring in redfish fillets were observed during the preobservation. All attributes for skinned and back bone side parameters were added in the preliminary scheme. Changes which were observed to occur in the skinned side and the backbone side parameters were almost the same as those included in the QIM scheme for deskinned redfish fillets parameters conducted by Githu (2013).

Changes were observed for skin and backbone sides of the fillets separately due to the presence of dark muscle which contains higher levels of lipids and myoglobin than the white muscles. Therefore, discoloration is presumed to be as a result of their oxidation during storage thus affecting the appearance of the fillets (Huss, 1995).

The description of gaping in the preliminary scheme was modified during the training sessions to describe more clearly the deepness of gaping apart from the split in the thick loin part. However, there was almost negligible changes in gaping over storage time. Flesh texture varied and depended on where in the fillet it was evaluated. Fillets tended to be firm at the loin part but soft at the tail. Therefore, detailed guidelines were given to panellists to always evaluate the texture at the same spot in the fillets for the sake of consistency (Martinsdottir, Sveinsdottir, Luten, Schelvis-Smith, & Hyldig, 2001).

During the preliminary training, an additional descriptor was added for appearance since the fillets appeared to develop a shiny mucus film on both sides with increase in storage period which was clearly seen and some changes in the selection of words were made to describe the changes in attributes more precisely. The preliminary scheme at the end of pre-observation contained two quality parameters, eight attributes and total quality index score of 22.

In the training sessions, panellist gave recommendations which resulted in the modification of attributes; colour, odour, gaping and appearance of mucus film on backbone side which became visible on last days of storage. Colour was a difficult parameter to evaluate since changes differ slightly with storage time, and odour since some fillets of same storage had a slight difference in odour hence new descriptors were included.

5.2 Shelf life study

5.2.1 Evaluation of raw fillets using QIM

In evaluation significant correlation (R^2 = 0.8156) was found between the panellists' total quality index score (sum of all attributes) and storage time after excluding texture and gaping attributes data (Figure 11b). This shows that the attributes gradually deteriorated with time and the results of quality index score increase linearly with storage time as recommended in the manual for sensory evaluation of fish freshness (Martinsdottir, Sveinsdottir, Luten, Schelvis-Smith, & Hyldig, 2001). Also, all individual attributes analysed indicated a clear linear relationship with storage time.

A minimum of three samples should be used due to individual variation present in the samples even from the same storage day (Bonilla, Sveinsdottir, & Martinsdottir, 2007). However, in freshness assessment guidelines for whole fish, it was recommended to use at least three (large fish) and ten (small fish) to study in the spoilage changes of whole fish (Martinsdottir, Sveinsdottir, Luten, Schelvis-Smith, & Hyldig, 2001). Also, it is recommended to use at least three whole fish samples to increase precision in prediction of storage time (Sveinsdottir, Hyldig, Martinsdottir, Jorgensen, & Kristbergsson, 2002).

The QI results indicate that the fillets reached a maximum point of rejection between 9 and 12 days, as study shows that spoilage sensory attributes begin to be highly detected from day 9. This was well supported by the results obtained from sensory evaluation of the cooked fillets (GDA).

However, there are other factors which must be taken into consideration which may affect the performance of the sensory panel. Since the thawed QIM scheme was new to the panel, it faced slight challenges as there was no prior experience evaluating with this scheme apart from the training sessions. Therefore, more training is needed for panellists so to reduce variation or wide distribution patterns in QI score for fillets of the same storage days which could also result in improvement of correlation and give more significant results with storage time.

5.2.2 Evaluation of cooked fillets using GDA

Redfish fillets reached the limits of acceptance between 9 and 12. End of shelf life is usually determined when spoilage related sensory attributes such as TMA, rotten odour and favour become evident (average score above 20) (Bonilla, Sveinsdottir, & Martinsdottir, 2007). Those odours and favours are mainly microbial origin (Huss, 1995). Based on the flavour scores in this study, the shelf life was 9 days, but juiciness of the fillet was not affected as it showed stable trend with storage days. Also, according to findings during the training session, fillets with 11 days of storage exhibited very strong spoilage characteristics and were deemed unfit for human consumption, hence it is likely maximum acceptability is 9 and 10 days of storage.

The end of shelf life was determined when the spoilage descriptive sensory score was above 20 and counts of TVC, H₂S producing bacteria were around 10^6 cfu/g and 10^5 cfu/g respectively. Based on this, the maximum storage time of the fillets was 9 days at temperature 1.7 ± 1.5 °C. This is a slightly longer shelf life than has earlier been reported for redfish fillets under traditional use of ice in processing, iced-stored (ice-ice) had a sensory shelf life of 4 days and when MAP packed (ice-MAP) had a shelf life of 8 days in cold room 0-2 °C (Lauzon, Stefansson, Jonsson, & Sveinsdottir, 2002).

5.2.3 Microbial counts

Microbiological results showed a similar growth curve for TVC and H_2S producing bacteria for the days in storage. The proportion of increase of H_2S bacteria was proportional to TVC over storage time. The fillet with 1 day storage sampled on second day of evaluation showed slightly higher TVC than the first fillet of the same storage time which had $1.3*10^4$ cfu/g and $2.7*10^4$ cfu/g respectively. There are no factors during the experiment that could explain the slightly different occurrence of bacteria counts, however, it could be related to prior handling during thawing procedures. In this study, the number of H_2S producing bacteria dominated the number of total viable counts especially from day 9 to last storage days, whereby TVC bacteria counts were 10^6 cfu/g and H_2S were 10^5 cfu/g on day 9 of storage.

The shelf life limit was reached at day 9 by showing TVC 6.90 log cfu/g which is approximately the maximum limit. The recommended upper limit for fish to be safe for consumption should not exceeded 7 log cfu/g (ICMSF, 2002). Therefore, microbial shelf life results seemed to correspond with sensory shelf life results of the red fish fillets. However as expected, bacteria growth occurred at a faster rate in fillets stored under aerobic environment than Modified atmosphere (MA).

6 CONCLUSION AND RECOMMENDATION

The QIM scheme developed for thawed red fish fillets in this study showed a linear relationship of total scores for quality attributes (QI) to storage time at 1.7 ± 1.5 °C. The QIM scheme consisted of eight parameters which gave a total of 22 points. The maximum storage time was estimated 9 days based on counts of TVC and H₂S-producing bacteria and sensory evaluation with GDA. The QI are therefore used to estimate the remaining storage time. At the beginning of the storage time, small changes in the positive attributes of odour and favour were observed. Starting from day 9 of storage they were hardly detectable, due to the increase of negative attributes. The QI could be used to estimate the remaining storage time on ice with an accuracy of ± 1.3 days if three fillets per lot were evaluated (Bonilla, Sveinsdottir, & Martinsdottir, 2007). This information may be used in quality management providing information about its quality and estimating remaining shelf life in thawed redfish fillets stored 1.7 ± 1.5 °C (assuming optimum storage conditions). However, differences among assessors indicated that the freshness assessment with the QIM scheme should preferably be based upon the assessment of more than one assessor.

During pre-observation trials of thawed whole herring findings, QIM has been shown to be a practical tool which could be developed to evaluate fresh fish for fish species in most African countries, but the number of attributes evaluated may vary based on species. Therefore, methods used in this study showed successful output of transferring and applying the methods in developing successful documented QIM manuals for various species in Tanzania and other African countries. Also, accurate estimation of shelf life using QIM method together with combination of GDA and microbial counts can play a great role in upgrading technical skills and it is recommended the development of manuals to support QIM in fish inspection and production quality assurance for even dried fishery products exported to neighbouring countries and local markets.

The need to use instruments required for steaming or boiling and a computerised system for the evaluation GDA results along with access to microbial laboratories may prove challenging in the East African environment where it would be necessary to develop alternatives. However, the manuals must contain the total plan for evaluation, explanation of the evaluation terms, and colour photos illustrating the different changing levels during the storage time of fish and products.

7 ACKNOWLEDGEMENTS

I would like to thank foremost my supervisor Kolbrun Sveinsdottir and panel leader Adalheidur Olafsdottir for the exceptional advice and guidance rendered to me, also Hildur Inga Sveinsdottir for her support during my study. I wish to express my gratitude to the MATÍS institute for support and I recognise the commitment of the sensory panel at MATÍS who were invaluable throughout my experiment. My gratitude to Dr. Tumi Tomasson, Programme Director of United Nations Fisheries Training Programme (UNU-FTP) and all the staff of UNU-FTP for their periodical review and comments on my project report which was most helpful. I am also grateful to all FTP Fellows who in one way or another assisted me during this project. Finally, I wish express my sincere appreciation to the Government of Tanzania-Kigoma Regional Office, UNU-FTP and the Government of Iceland who made my participation in UNU-FTP 2017 possible.

8 LIST OF REFERENCES

- Alasalvar, C., Taylor, K. D., Oksuz, A., Garthwaite, T., Alexis, M., & Grigorakis, K. (2001). Freshness assessment of cultured sea bream (Sparus aurata) by Chemical, physical and sensory methods. *Food chemistry*, 72(1), 33-40.
- Bonilla, A., & Sveinsdottir, K. (2005). Development of Quality Index Method (QIM) scheme for fresh cod. *Food Control*, 18(4), 352-358.
- Bonilla, A. C., Sveinsdottir, K., & Martinsdottir, E. (2007). Development of quality index method (QIM) scheme for fresh cod (Gradus morhua) fillets and application in shelf life study. *Food control*, 18, 252-258.
- Bonilla, A. C., Sveinsdottir, K., & Martinsdottir, E. (2007). Development of quality index method (QIM) scheme for fresh cod (Gradus morhua) fillets and application in shelf life study. *Food control*, 18, 252-258.
- Botta, J. (1995). Evaluation of Seafood Freshness Quality. New York: VCH Publishers, Inc.
- Burt, J. R., & Hardy, R. (1992). Composition and deterioration of pelagic fish. In Pelagic fish: The resource and its exploitation. Fishing News Books-Division of Blackwell Scientific Publications Ltd.
- Capell, C. V. (1997). Use of counts of hydrogen sulphide producing bacteria to estimate remaining shelf life of fresh fish. *Proceedings of the final meeting of the concerted action "evaluation of fish freshness"*, pp. 175-182.
- Church, N. (1998). Feature MAP fish and crustaceans-sensory enhancement. *Food*, *12*(2), 73-82.
- Daun, H. (1993). Introduction in Charalambous G, Shelf life studies of foods and beverages, Chemical, Biological, Physical and Nutritional Aspects. Amsterdam: Elsevier science Publishers B V.
- Fisheries. (2017, November). *Iceland responsible fisheries*. Retrieved from responsible fisheries: http://www.responsiblefisheries.is/files/golden-redfish
- Fisheries, D. (2015). *Fisheries annual statistical report*. Dar es Salaam, Tanzania: Ministry of livestock and fisheries development.
- Githu, W. N. (2013). Development and application of quality index method (QIM) in quality determination and shelf life study of Red fish fillets: The effect of bleeding, superchilling and packaging in modified atmosphere. Iceland: United Nations University- Fisheries Training Programme.
- Gram, L., & Huss, H. (1996). Microbial spoilage of fish and fish product. *International Journal* of Food Microbiology, 33, 121-137.
- Huss, H. H. (1995). *Quality and quality changes in fresh fish*. FAO. Rome: FAO Fisheries Technical Paper.
- Hyldig G, N. J. (1997). A rapid sensory method for quality management. *Proceedings of the final meeting of the concerted action "evaluation of fish* (pp. p. 297-305). IIR.

- Hyldig G., N. J. (2004). QIM- A Tool for Determination of Freshness. In G. N. Hyldig, & B.
 K. Fereidoon Shahidi (Ed.), *Seafood Quality and Safety: Advances in the New Millennium* (p. 381). St. John's, Newfound land, Canada: Science Tech Publishing Company.
- Hyldig, G., Bremer, A., Martinsdottir, E., & Schelvis, R. (2007). Qulity Index Methods. In *Handbook of meat, poultry and seafood* (pp. 529-49). Oxford,UK: Blackwell Publishing.
- ICMSF. (2002). International Commission on Microbiological Specifications for Foods. Microorganisms in foods 7 – Microbial testing in food safety management. New York.: Kluwer Academic/Plenum Publishers.
- Lauzon, H. L., Stefansson, G., Jonsson, G. P., & Sveinsdottir, K. (2002). Sensory quality and shelflife of MAP Redfish (Sebastes marinus) fillets as affected by previous MA bulk storage of whole fish. *Aquatic food product technology*, 11, 251-273.
- Lawless, H., (1998). Sensory Evaluation of Food: Principles and Practices. New York: Kluwer Academic/Plenum Publishers.
- Liston J. (1980). Advances in fishery science and technology. In *Microbiology in fishery science* (pp. 138-157). Farnham, England: Fishing News Books Ltd.
- Magnússon, H., Sveinsdóttir, K., & Þorvaldsson, L. (2010). The effect of different cooling techniques on the quality changes and shelf life of whole cod (Gadus morhua). p. 28.
- Martinsdottir, E., Sveinsdottir, K., Luten, J., Schelvis-Smith, R., & Hyldig, G. (2001). *Sensory* evaluation of fish freshness. Referce manual for fish sector, Netherlands.
- Meilgaard, C. M. (1991). *Sensory Evaluation Techniques* (Fifth Edition ed.). Boca Raton: CRC Press.
- Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007). *Sensory Evaluations Techniques* (4th ed.). Boca Raton, : CRC Press.
- Murray, J. M. (2001). Descriptive Sensory Analysis: Past, Present and Future. *Food Research International*, *34*(6), 461-471.
- Nielsen, D., & Hyldig, G. (2004). Influence of handling procedures and biological factors on the QIM evaluation of whole herring (Clupea harengus L.). *Food Research International*, *37*, 975–983.
- Oehlenschlager, J., & Sorensen, N. (1997). *Criteria of seafish freshness and quality aspects in seafood*. International institute of refrigeration. Nantes conference.
- Oehlenschlager., J. (2002). Influence of different pre-storage treatments on resulting shelflife of iced-stored barent-sea cod. *Aquatic food production technology*, *11*, 187-200.
- Olafsdottir, G. F. (1997). Evaluation of fish freshness using volatile commpound in fish. *Proceedings of the final meeting of the concerted action "evaluation of fish freshness*, pp. 55-69.
- Olafsdottir, G., Lauzon, H., Martinsdottir, E., & Kristbergsson, K. (2006). Influence of storage temperature on microbial spoilage characteristics. *International journal of food microbiology.*, 111(2), 112-125.

- Olafsdottir, G., Martinsdottir, E., Oehlenscbger, J., Dalgaard, P., Jensen, B., Undeland, I., & Nilsen, H. (1997). Methods to evaluate fish freshness in research and industry. *Trend in food science and technology*, 8(9), 258-265.
- Rodríguez-Jérez, J., Hernández-Herrero, M., & Roig-Sagués, A. (2004). New methods to determine fish freshness in research and industry. *Global quality assessment in Mediterranean aquaculture*, 63-69.
- Saborido-Rey, F., Garabana, D., Stansky, C., Melnikov, S., & Shibanov, V. (2005). Review of the population structure and ecology of S. mentella in the Irminger sea and adjacent waters. *Review in fish biology and fisheries*, *14*(4), 455-497.
- Stone, H., & Sidel, J. (2004). Sensory Evaluation Practices. London: Elsevier Academic Press.
- Stransky, C. (2005). Geographic variation of golden redfish (Sebastes marinus) and deep-sea redfish (S. mentella) in the North Atlantic based on otolith shape analysis. *Journal of Marine Science*, 62(8), 1691–1698.
- Stroud, G. D. (2001). *The Herring. Torry Advisory Note No. 57.* Ministry of Agriculture, Fisheries and Food , Torry Research Station.
- Sveinsdottir, K., Hyldig, G., Martinsdottir, E., Jorgensen, B., & Kristbergsson, K. (2002). Application of quality index method (QIM) scheme in shelf life study of farmed Atlantic salmon(Salmo salar). *Journal of Food Science*, 67(4), 1570-1579.
- Sveinsdottir, K., Hyldig, G., Martinsdottir, E., Jorgensen, B., & Kristberrgasson, K. (2003). Quality Index Method (QIM) scheme development for farmed Atlantic salmon (Salmo solar). *Food Quality and Preference*, 14, 237-245.
- Sveinsdóttir, K., Martinsdóttir, E., Green-Petersen, D., Hyldig, G., Schelvis, R., & Delahunty, C. (2009). Sensory characteristics of different cod products related to consumer preferences and attitudes. *Food Quality and Preference*, 20(2), 120-132.

9 APPENDIX

Quality	Description	Score
parameter		
	Firm	0
Texture	Rather soft /Fairly firm	1
	Soft	2
	Very soft	3
	Fresh, marine	0
	Cold room, starchy	1
Odour-skin side	sour, rancid at tail part	2
	Very sour, putrid, rotten	3
	Fresh, marine	0
Odour-Back	Cold room, starchy	1
born side	Sour	2
side	Very sour, putrid, rotten	3
	Translucent, bluish	0
Colour -	Slightly milky with slightly brown at the edges	1
backbone side	Milky, slightly yellowish at the edges	2
	Yellowish, greenish at the edges part	3
	Bright, light brown	0
Dark muscle Colour-	Brown reddish, less toward the lean part, slightly Brownish	1
Skin side	Brown-greyish, slightly yellow at the edges	2
	yellowish-greenish at the edges	3
	No gaping, one gaping in the thick loin or neck part of the fillet	0
Ganing	Slight gaping, flesh torn less than 25 %	1
Suping	More gaping, flesh torn 50-75 %	2
	Deep gaping over 75%, flesh more torn	3
Quality Index (0-	18)	

Appendix 1: Preliminary QIM Scheme for thawed deskinned redfish (Sebastes marinus) fillets

Appendix 2: Preliminary QIM scheme for sensory evaluation of whole thawed herring (C	Iupea
harengus).	

Quality pa	arameter	Description	Score
i		Silvery-shiny skin	0
	Colour/	Skin is less shiny	1
	appearance	Matt, dull mainly near the abdomen	2
		Dull slight vellowish at lower part of the abdomen	3
		Hard	0
	Texture of loin part	Firm with finger mark disappear after a second	1
Whole		Yield	2
fish	The form of	Firm	$\overline{0}$
	holly	Soft	1
	berry	Very soft, burst	2
		Fresh sea	0
	Odour	Neutral	1
	Ououi	Fermentation remains, slightly off	2
		Strong off odour	3
	D '1	Clear and black, metal shiny	0
	Pupils	Opaque	1
		Grey	2
Euco	Compa	Clear Opelescent reddish	0
Eyes.	Cornea	Milky red brownish	$\frac{1}{2}$
		Convex	
	Shape	Flat	1
	Shape	Concave, sunken	$\frac{1}{2}$
	Colour/ appearance	Bright red	0
		Slightly red, pinkish	1
		Brownish	2
Gille		Grey-brown, green	3
UIIIS	Odour	Fresh, seaweed, metallic	0
		Neutral, grassy	1
	Ououi	Sour grassy, mouldy, yeast, fermentation remains	2
		Strong off odour, rotten	3
		Fresh, intestinal organs are in good shape	0
	Appearance	Slightly pale, very delicate organs slightly	1
	content	More pale intestinal organs digested by enzymes	
	content	brownish thick mucus	2
	DI 11	Blood red/not present	0
Abdomen	Blood in	Dark red	1
	abdomen	Blood more brownish	2
		Neutral	0
	0.1	Cucumber, melon, table cloth	1
	Udour	Sour, reminds of fermentation, rancid	2
		Rotten/rotten cabbage,	3
Quality In	dex (0 – 29)		



Appendix 3: Pictures of panellists at QIM and GDA training sessions

