UNITED NATIONS UNIVERSITY
Fisheries Training Programme

unuftp.is Final Project 2012

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

Winnie Ng'endo Githu
Ministry of Fisheries Development
Mombasa, Kenya
w.ngendo@yahoo.com

Supervisors:
Kolbrún Sveinsdóttir
Matís
kolbrun@matis.is

Emilia Martinsdóttir
Matís
emilia@matis.is

ABSTRACT

Effective quality control management by fish processing industries and fish inspection demands fast and reliable freshness evaluation methods such as Quality Index Method (QIM). The aim of the study was to develop a QIM scheme for deskinned redfish fillets and evaluate it in a shelf life study of air (A) and Modified Atmosphere Packaged (MAP): 40% CO_2 and 60% NO_2 (M1) and 40% CO_2 and 60% Ar (M2) redfish fillets. Gas compositions for the MAP were identified in a preliminary study. The effects of gas mixture, bleeding, and storage temperature on the shelf life and quality parameters were evaluated during superchilled (-0.5°C; day 0- 6) and chilled (2°C; day 7-16) storage. The changes during storage time were assessed by measuring drip, sensory evaluation of raw fillets using QIM and of cooked fillets using Torry-scheme and Quantitative Descriptive Analysis (QDA), and total viable microbial counts (TVC). The results of the shelf life study showed no significant difference in quality changes between the MAP fillets (M1 and M2). However, difference in MAP compared to the air packaging was found significant for all the analysis. Maximum storage time was 11 days for A and 14 days for MAP (M1 and M2) in superchilled storage. Quality index (QI) scores were around 8 for all the sample groups at sensory rejection point evaluated by Torry scheme. This implied that the developed QIM scheme would be applicable for evaluation of MAP as well as air packaged fillets.

This paper should be cited as:
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*. United Nations University Fisheries Training Programme, Iceland [final project]. http://www.unuftp.is/static/fellows/document/win12prf.pdf

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

Fisheries sector is an important production sector for high protein food in many countries. Trade in fish also represents a significant source of foreign currency earnings, employment and income generation. In recent years, global consumption of seafood products has risen dramatically and products present in the fish market have evolved considerably. About 131 million tonnes from world capture and aquaculture fisheries was destined for human consumption in 2011 (FAO 2012).

Previously presented as whole fish, technological developments in fish processing and packaging has led to an increase in number of processed products that are now offered to consumers. Fish and fishery products are generally distributed as live, fresh, chilled, frozen, heat-treated, fermented, dried, smoked, salted, pickled, boiled, fried, freeze-dried, minced, powdered or canned, or as a combination of two or more of these forms. However, variations in utilization and processing are significant among continents, countries and within countries (Ababuoch 2010).

Owing to the high perishability, quality and shelf life of fish and fishery products are a major concern to fish industry and consumers. Handling practices and storage conditions are the most important factors that affect the quality and shelf life of fish and fishery product (Huss 1995). To ensure high quality products on the market, it is of the essence to maintain the freshness of the fish and fishery products by maintaining cold chain, providing optimal handling and transport conditions (Olafsdottir *et al.* 2006). Sensory evaluation is an important method for the assessment of freshness and quality, and is commonly used in the fish sector and fish inspection services (Olafsdottir *et al.* 1997).However correlation of sensory quality with microbial and chemical quality parameters is important in determining the quality and shelf life of seafood.

Frozen and fresh redfish form a significant part of the seafood exported from Iceland to international markets mainly Europe and USA. The largest segment of fresh fish exports is as whole fish that is transported by container ships. However, the market for fresh fillets in the European market has grown quite rapidly in the past years, fuelled by the rise in their prices and increase in demand. The fillets are processed soon after landing from the vessels, graded, packed, chilled and then transported by air to European market to maximize freshness and shelf life (Icelandic Fisheries 2012). Freshness evaluation is of the essence at different transaction points in the production and distribution chain in order to ensure quality products in the end market hence the need to apply fast and reliable freshness assessment methods such as Quality Index Method, QIM, (Martinsdottir 2002).

Limited shelf life of fresh fish products is a large hurdle for the export of fresh products from Iceland to mainland Europe or USA (Magnússon *et al.* 2010). Temperature control is a major concern during fresh fish distribution by airfreight especially before loading and after unloading. Minimal deviation from the optimal storage temperature is registered in sea freights. Extension of fresh fish shelf life would enhance use of sea freight for transport of fresh fish products from Iceland. Studies have shown that superchilling and MAP extends shelf life of seafood. However, effectiveness of MAP is dependent on the quality of the raw material, gas combination applied and storage conditions. Different gases have varying impact on sensorial qualities of fish products hence the need to study the effect of MAP on shelf life of superchilled redfish fillets.

The fisheries sector plays an important role in the Kenyan national economy and supports about 80,000 fishers directly and about 800,000 individuals (processors, traders and other service providers) indirectly(GOK FID 2008). Production comes from both aquaculture and capture fisheries comprising of marine and freshwater (inland lakes and rivers).It is believed that higher contribution to the national gross domestic product by fisheries sector from the current 5% can be realized through transformation in the sector that entails growth in competitive trade and diversification of fish value added products. The potential can be achieved through provision of high quality products in the local and international markets, which can earn premium prizes. This will lead to food security and higher income.

There is a considerable variation in methods of fishing and fish handling on board fishing vessels and on landing beaches. Fishing is mainly artisanal with wide range of fishing boats mostly comprised of dugout and outrigger canoes, dhows, and larger sailing ships. Most of the crafts are non-motorized with sails (43%) and paddles (40%) as the forms of propulsion for the crafts(GOK FID 2012). Exposure of un- iced fish to ambient temperatures and lack of insulated containers are main fish handling characteristics at artisanal level. Commercial fishing vessels are fitted with refrigerated and insulated fish holds or ice boxes. Exposure of fish to high ambient temperatures and lack of proper cooling structures, leads to rapid quality deterioration. This imparts negatively on the overall quality of fish destined to the markets.

Fish inspectors are charged with the responsibility of ensuring safety and quality of locally and internationally traded fish and fish products. This involves inspection of fish and fish handling methods at the production, transport, markets and fish processing factories in order to ensure compliance with national regulations (GOK FID 2007) and international regulations notably the EU regulations governing food safety and quality.

Fish quality inspectors at, receiving points in processing factories and fish markets are relying on traditional sensory methods for freshness evaluation. Application of technology such as Modified Atmosphere Packaging (MAP) of fresh fish, which would result in extending shelf life of fish and fish products with high economic benefits, is minimally utilized. Development of fast and reliable freshness evaluation method such as QIM, for fish species found in the Kenyan waters and its application in the fishery sector is essential especially in the quality control management by fish processing industries and fish inspectors. QIM will be useful to give feedback to the fishermen concerning the quality of their catch, which may in turn influence better handling on board.

The aim of this study was to develop a QIM scheme for deskinned red fish fillets and use the scheme during a shelf life study to estimate freshness of red fish fillets stored at temperatures simulating sea freight transport and retail storage redfish fillets in modified atmosphere during storage. Further, the aim was to study the QIM results in relation to other sensory methods (Torry and Qualitative Descriptive Analysis), microbial, chemical and physical methods for estimation of the shelf life of the fillets and select the appropriate MAP gas composition. The aim was also to gain experience in different quality evaluation methods and learn how to train and use panellists in sensory evaluation. In addition, the aim was to gain knowledge on effects of different fish handling, packaging and storage conditions such as temperature control, bleeding, MAP and superchilling on quality and shelf life of seafood.

2 LITERATURE REVIEW

2.1 Freshness changes in stored fish

Freshness is a property of fish that has considerable influence on quality. For all kinds of products, freshness is essential for the quality of the final product (Olafsdottir *et al.* 1997). According to (Olafsdottir *et al.* 1997), freshness can be explained to some extent by some objective sensory, biochemical, microbial and physical parameters. Performing controlled experiments to denote the parameter descriptors can monitor freshness and spoilage.

Soon after fish is caught, freshness of the fish starts to decline and progresses until the fish is no longer acceptable for consumption. Characteristic post mortem changes are easily perceived with human senses. The first changes are concerned with appearance and texture (Huss 1995). Immediately after death, fish muscle becomes hard and stiff and is said to be in *rigor mortis*. When rigor resolves, fish muscle relaxes and becomes limp. According to Huss (1995) onset, length and resolution of rigor are influenced by temperature, time, handling, size and physical condition of the fish. *Rigor mortis* indicates better quality and freshness of the fish (Rodríguez-Jérez *et al.* 2004).

Enzymatic autolysis, oxidation, and microbial growth in the fish muscle results to a characteristic four phase spoilage pattern (Huss 1995). Spoilage manifests itself as changes in the sensory characteristics in the four phases. There is little deterioration in the first phase and the fish is described as very fresh with slight loss of characteristic odour and flavour. The fish losses the natural flavour, odour and becomes neutral in the second phase. Signs of spoilage associated with textural changes, strong off-flavours and odour begin to show in the third phase due to increase in spoilage bacteria as storage progresses. Sulphur and nitrogenous volatiles are detectable and the fish is evaluated as unfit for human consumption. In the final phase, the fish is described as spoiled and putrid.

The rate of deterioration during storage varies with fish species and depends on biochemical compositions of the substrates and metabolites in the fish tissue, microbial contamination, fish handling and the condition of storage (Masniyom 2011).

2.2 Shelf life of fresh fish

Huss (1995) describes shelf life of fish as the time from when the fish is caught until it is no longer fit for consumption. Under normal aerobic chilled storage, the shelf life of fish and fish products is limited by the growth and biochemical activities of aerobic specific spoilage organisms (SSO). However, enzymatic and microbiological activity is greatly influenced by temperature; therefore the shelf life of fish and fish products is markedly extended by cooling to a temperature approaching that of melting ice (chilling). Research studies have been carried out relating shelf life and storage temperature of various fish species. According to Magnússon *et al.* (2010) freshness loss in iced, whole fish depends on species and ambient temperature, being shortest for cod (7-9 days) in comparison to American plaice (10 days) and redfish (14 days).Other intrinsic factors as outlined in table 1, affect spoilage of fish in ice.

Table 1: Intrinsic factors affecting spoilage of fish in ice (Huss 1995).

Factors affecting spoilage rate	Relative spoilage rate	
	Slow rate	**Fast rate**
Shape	Flat fish	Round fish
Size	Large fish	Small fish
Fat content in the flesh	Lean species	Fatty species
Skin characteristics	Thick skin	Thin skin

The limited and variable shelf life of chilled fish products is a major problem for their quality assurance and commercial viability. Earlier study by Magnusson & Martinsdottir (1995) described shelf life of fresh cod fillets (processed one day post catch) in ice as 10–12 days. However later study by Cardenas Bonilla *et al.* (2007) showed a shelf life of 8-10 days for cod fillets processed 3 to 5 days post catch. The length of time before processing influenced the shelf life of the fillets. Apart from hygienic handling and processing, and chilling, studies have shown that superchilling and MAP extends shelf life of seafood (Sivertsvik *et al.* 2003,Wang *et al.* 2008).

2.2.1 Effects of Superchilling on Shelf life

Huss (1995) defines superchilling or partial freezing as storage of fish at temperatures between 0°C and -4°C. It is based on lowering core temperature of the fish close to freezing point (sub-zero state), which depends on water content and soluble substances of fish. Lag phase of microbial growth is extended at the beginning of storage and spoilage is reduced resulting in extended shelf life (Huss 1995).

Various types of cooling systems have been used for superchilling of seafood products including superchilled brine, slurry or liquid ice (small ice crystals in superchilled brine) and dry ice (CO_2 in solid form), depending on fish species, processing stages and products, flake ice or slurry ice (Bao *et al.* 2007). Research studies have shown that superchilling extends the shelf life of fish products. Superchilling of Arctic charr fillets at -2°C of fillets packed with dry ice resulted in 6 days shelf life extension compared to chilled (3°C) fillets (Bao *et al.* 2007). According to (Ólafsdóttir *et al.* 2012) shelf life of superchilled cod fillets was estimated to be between 16 to 18 days compared to 10-12 days in ice (Magnusson and Martinsdottir 1995). Research study by (Olafsdottir *et al.* 2006) using Combined Blast and Contact (CBC), a new superchilling technique showed higher shelf life extension for cod fillets to at least 15 days at -1.5°C based on Torry score and TVB-N compared to 11 days in ice storage.

Superchilling may have negative effects on freshness and prime quality of the fish products from different fish species. Cod stored at -2°C for 10 days had an appearance and texture inferior to fish stored at 0°C in ice and at -3°C drip was increased (Huss 1995). The negative effects on drip loss, appearance, and texture of cod are due to formation of large ice crystals, protein denaturation and increased enzymatic activity in the partially frozen fish (Huss 1995) and (Bao *et al.* 2007). During partial freezing some of the water freezes out and the concentration of solutes in unfrozen solution increases. This may lead to denaturation of the muscle proteins as well as structural damage of membranes, which can result in increased drip loss and textural changes (Duun and Rustad 2007).

2.2.2 Effect of Modified Atmosphere Packaging on shelf life

Packaging protects of fish and fish products against deteriorative (microbial, biochemical, and physical) effects (Masniyom 2011). Replacing the normal composition of air in the pack with a single gas or a mixture of gases can modify the atmosphere surrounding a fish product in a package. However, effectiveness of MAP depends on initial microbial quality, species, gas mixture, gas to product volume ratio, packaging materials and storage temperature (Wang *et al*., 2008, Sivertsvik *et al.* 2002). The three main commercially used gases in modified atmosphere packaging are: carbon dioxide (CO_2), nitrogen (N_2) and oxygen (O_2). CO_2 is soluble is soluble in water and lipids and therefore the most important gas due to its bacteriostatic and fungistatic properties (Huss 1995,Sivertsvik *et al.* 2002)). Excessive absorption of CO_2 results to "package collapse". High CO_2 content lowers the pH and consequently causes increase in dripping due to low protein water retention of the fish muscle (Huss 1995, Masniyom 2011). Nitrogen replaces oxygen in packaging and thereby prevents oxidation of lipids. Owing to its low solubility in water, N_2 also helps to prevent package collapse by maintaining internal volume. Argon replaces oxygen better than N_2. Studies on Argon modified atmosphere have shown positive effect on the preservation and shelf life of trout fillets (Choubert *et al.* 2008) .Presence of oxygen may cause oxidative rancidity problems in fish presenting high lipids therefore in MAP low concentration of O_2 retards the growth of aerobic microorganisms and reduces the degree of oxidation (Soccol and Oetterer 2003).

Various research studies have explained the effect of different gas combinations on shelf life of packed fish products (Table 2).

Table 2: Shelf life extension of fish and fishery products in MAP.

Fish and fishery products	Storage temperature (°C)	MAP condition CO_2:O_2:NO_2	Shelf life (days)		References
			air	MAP	
Mediterranean swordfish (*Xiphias gladius*)	4	40:30:30	7	12	(Pantazi *et al.* 2008)
Pearlspot (*Etroplus suratensis* Bloch)	2	60:40:0	11	21	(Ravi Sankar *et al.* 2008)
Sea bass (*Dicentrachus labrax*)	4	60:10:30	6	13	(Kostaki *et al.* 2009)
Atlantic salmon (*Salmon salar L.*)	2	90:0:10	11	22	(Fernández *et al.* 2009)
Sea bass (*Dicentrachus labrax*)	4	60:0:40	7	14	(Provincial *et al.* 2010)

Study by Wang and others 2008 on synergistic effect of MAP and superchilling of cod loins showed extended shelf of 21 days compared to superchilling (16 days) and modified atmosphere (14 days) alone.

2.3 Effects of bleeding of fish quality

Bleeding is carried out to eliminate blood from the blood from the tissues (Maqsood and Benjakul 2011). However, the process should be carried out immediately after catch in order to maintain optimal quality in the fillets (Huss 1995). Borderías and Sánchez-Alonso (2011) reported that bleeding in Atlantic salmon is only effective if conducted within 1 to 2 hours of capture. Improper bleeding influences visual appearance of the fillets characterized by residual blood in the fish tissue. Residual blood induces development of undesirable odour and flavour of the fillets that lowers marketability of fish (Maqsood and Benjakul 2011, Roth *et al.* 2005). Bleeding removes heme proteins that accelerate oxidative rancidity in the fish muscle. Study by Maqsood and Benjakul (2011) showed that bleeding effectively lowered heme proteins in Asian seabass fillets. Richards and Hutlin (2002) also reported that bleeding reduced rancidity in minced trout whole muscle, minced mackerel light muscle, and intact mackerel dark muscle.

2.4 Methods for evaluation of fish freshness and quality

2.4.1 Sensory Evaluation methods

Sensory evaluation is considered as the principal method to evaluate the freshness of seafood. The analysis method involves evaluation of food sensory attributes using the human senses. In the fisheries sector, sensory evaluation of fish freshness gives valuable information on fish freshness and quality based on appearance, odour, flavour and texture parameters of the fish. Subjective and objective methods are applied in sensory evaluation. Subjective methods are based on panellist's preference and acceptability of a product therefore bias may exist among the assessors. This method is often applied market research studies and product development (Huss 1995).

In fish quality evaluation, objective descriptive tests are applied using a group of trained panellists (Martinsdottir *et al.* 2009). Structured scales describing the nature intensity of the quality parameters are used. Different grading schemes have been developed for sensory evaluation of whole and cooked fish. EU schemes and Quality Index Method (QIM) are used in freshness evaluation of raw whole and gutted fish while Torry scheme and Quantitative Descriptive analysis (QDA) are employed in evaluation of cooked fish.

Europe recommends use of EU scheme for quality assessment of raw fish in the industry and inspection service according to the Council Regulation (EC) No. 2406/96 of November 26, 1996 (Anonymous 1996). In the scheme, general parameters are used to describe the freshness without taking into account variation of different fish species. Improved and more accurate evaluation methods have been developed.

Quality Index Method (QIM)

Inaccuracy of the EU scheme led to development of new, accurate and objective seafood freshness grading system that takes into account differences between fish species. Different fish species show different sensory changes during spoilage. QIM is therefore based on well-defined characteristic changes that occur in raw fish (Martinsdóttir *et al.* 2001). A score of 0-1, 0-2 or 0-3 demerit (index) points is given for changes occurring in odour, texture and external appearance of gills, skin and eyes as listed in the grading scheme. Zero (0) score corresponds to very fresh fish

and consequential increase in the score occurs as the freshness declines. The scores for all the characteristics are summarized to give an overall sensory score called the Quality index (QI). QIM schemes have been developed for various fish species. The information is available online at QIM Eurofish website (http://www.qim-eurofish.com). Recently, QIM scheme for bogue (*Boops boops*, *L*.) and blackspot seabream (*Pagellus bogaraveo*) have been developed. (Bogdanović *et al.* 2012, Sant'Ana *et al.* 2011). Increasing interest in use of QIM as quality assessment tool for processed fish products has led to development of QIM for raw fish fillets. Sensory changes on the fillets are specific to different fish species. Descriptors for colour, odour, texture, gaping and appearance or any other important parameter depending on particular fish species are listed in the scheme. Cardenas Bonilla *et al.* (2007) described QIM scheme for fresh cod (*Gadus morhua*) fillets. Further research is needed to evaluate applicability of QIM for fish handled, stored and processed under different conditions. It's known that different cooling techniques have different influence on sensory attributes not always correlated with spoilage hence the need to study influence of superchilling and MAP on sensory qualities of redfish fillets and develop a QIM suitable for future quality assessment in this storage condition.

The aim of QIM development is to have QI increase linearly with storage time in ice (Martinsdóttir *et al.* 2001). Under well-controlled experiments, QIM can be used to estimate shelf life and remaining shelf life of fish and fish products. The number of days that whole, fresh (gutted) fish is stored in ice until it becomes unfit for human consumption gives the shelf life while predicted storage time in ice is the number of days that the fish has been stored in ice. Estimate of the remaining shelf life (shelf life–predicted storage time) is therefore determined (Martinsdóttir *et al.* 2003, Hyldig and Green-Petersen 2004). In QIM development, experienced panellists develop best descriptors for the spoiling fish, which also fulfil the aim that it is possible to predict the remaining shelf life for the species. The drafted QIM is finalized by testing it in a shelf life study and the same time defining the shelf life of the fish (Hyldig *et al.* 2007). Use of photographs in QIM development clearly illustrates changes of descriptors in the different attributes therefore it can easily be used in training people inexperienced in fish evaluation and quality inspectors in the fish industry. Good sensory evaluation results depend on use of trained panel and guidelines for assessment. According to Martinsdóttir *et al.* (2001), assessment should preferably be carried out by more than one trained assessor using a QIM scheme. All attributes from a homogenous lot should be assessed in the same order for each fish.

Research has provided an objective and powerful tool that can be implemented at different levels in the fishery chain. Implementation of QIM at fish auctions provides information on fish quality therefore it is important for effective quality and process management in production of high quality fish products. Recent development in the implementation of QIM in Europe has led to development of QIM software programme that enables evaluation of fish freshness using supportive illustrative photographs of specific parameters and provides remaining shelf of fish (Figure 1). Its application has enabled quality evaluation of 13 fish species (Pollock, haddock, herring, turbot, sole, brill, redfish, deep water shrimps, fjord shrimps and peeled shrimps, salmon, cod and plaice) based on the QIM described in QIM manual (Martinsdóttir *et al.* 2001). Martinsdóttir *et al.* (2003) recommended further research in application of QIM for fish stored under different conditions since most of the already existing QIM schemes are based on controlled chilled storage studies.

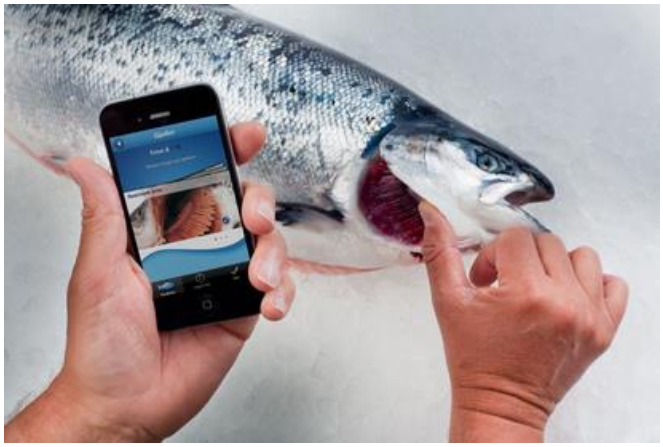


Figure 1: Application of QIM software in quality evaluation of fish (QIM Eurofish 2012).

Torry Scheme

Freshness of fish fillets can also be determined through evaluation of sensory parameters in cooked fillets. Sensory evaluation of cooked fish fillets involves evaluation of flavour, odour and texture by a sensory panel. Modified Torry scale, based on original work by the Torry Research Station (Shewan *et al.* 1953) is used for the evaluation. A descriptive scale of 10 points has been developed for lean, medium fat and fatty fish species. Scores are given from10 (very fresh in taste and odour) to 3 (spoiled) and average score of 5.5 as the limit for consumption due to detection of off flavour and odour attributes related to spoilage (Martinsdóttir *et al.* 2001). Descriptions below 3 are considered unnecessary as the fish is then considered unfit for consumption. It is therefore possible to determine maximum storage time of fish through sensory evaluation of cooked fish samples

Quantitative Descriptive Analysis (QDA).

QDA principle is based on the ability to detect and describe quantitative sensory attributes of a product by a trained panel of (Stone and Sidel 2004) under guidance of a panel leader. The method has been used on cooked fish samples to determine the maximum storage time and give detailed description of the sensory profile of the fish (Sveinsdottir *et al.* 2003) .In QDA an unstructured 15 cm (6 inches) line scale(0-100%) is used. Panellists indicate the relative intensity of the attribute by making a mark on the line. The scale direction is always from left to right with increasing intensity for example weak to strong or light to dark. Terminologies to be used during evaluation are first developed during an interactive session with guidance from the panel leader. However, the panel leader does not participate in developing the terminologies but facilitates the communication process. Furthermore, those subjects who have technical knowledge of products should not be invited to participate sensory terminology development in order to avoid possible bias in the results (Stone and Sidel 2004). Panellists are then trained to familiarize with the attributes and definitions developed using the unstructured scale. Finally, the panellists participate in the evaluation process that is carried out in separate booths to reduce distraction and panellist interaction (Meilgaard *et al.* 1991). The overall success of using the QDA in evaluation is highly dependent on the commitment and the motivation of the panellists irrespective of their potentiality therefore it is important to assess the availability of the panellists through individual interviews (Murray *et al.* 2001). Apart from application of QDA in shelf life studies without dependence on standards or control products, the method is also applied in monitoring competition of products,

product development and in relating instrumental and sensory analysis methods (Stone and Sidel 2004).

2.4.2 Non-sensory analysis

Changes in seafood during storage due to microbial activity, autolytic enzymes or chemical reactions can be useful indices of quality or spoilage. During fish spoilage, there is breakdown of proteins in the fish muscle and the formation of new compounds that are responsible for the changes in odour, flavour and texture of the fish muscle. Early deterioration process is enhanced by endogenous proteases. Hansen *et al.* (1996) reported that autolytic enzymes reduced textural quality during early stages of deterioration but did not produce the characteristic spoilage off-odours and off-flavours. This indicates that autolytic degradation can limit shelf-life and product quality even with relatively low levels of spoilage organisms. According to Huss (1995),autolysis is accelerated by physical handling of fish. Composition of the micro flora on newly caught fish depends on the microbial contents of the water in which the fish live. Microbial growth and metabolism is a major cause of fish spoilage. Each fish product has its own specific spoilage bacteria related to the shelf life. Psychrotolerant gram-negative bacteria (such as *Pseudomonas spp*. and *Shewanella spp*) cause deterioration of chilled fish. Spoilage bacteria are responsible for production of amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones responsible for the unpleasant and unacceptable sensory off-flavours. Olafsdottir *et al.* (2006) reported on the proliferation of specific spoilage organisms in haddock fillets stored at 0,7 and 15°C and found *Photobacterium phosphoreum* to be predominant among spoilage bacteria. *Pseudomonas spp.* appeared responsible for sweet, fruity spoilage odours while *Shewanella putrefaciens* was responsible for the H_2S production. Measurement of bacteria counts and volatile compounds responsible for the sensory changes during spoilage can be used to evaluate fish freshness and spoilage parallel to the sensory evaluation.

3 MATERIALS AND METHODS

3.1 Experimental design

Figure 2 shows the organization of the whole study into preliminary and main study. The preliminary study focused primarily on development of a Quality Index Method (QIM) scheme for deskinned red fish fillets by monitoring evolution of sensory characteristics of vacuum (V), modified atmosphere M1 (40% CO_2: 60% Ar), M2 (40% CO_2: 60%N_2), M3(60% CO_2: 40% Ar), M4(60% CO_2: 30%Ar: 10%N_2) and air stored fillets (A) during superchilled storage (0 to -5°C). The aim was to identify, the optimal gas mixture to be used in a shelf life study (main study) Evaluation of raw fillets (n=5 per sample group) was done on day 0, 5, 11, 13 and 20. Drip loss of fillets from each sampled tray was determined on day 6 and 11 while the cooked fillets (n=4 per sample group) and headspace gas composition in each sampled tray were analysed on day 0, 6 and 11.

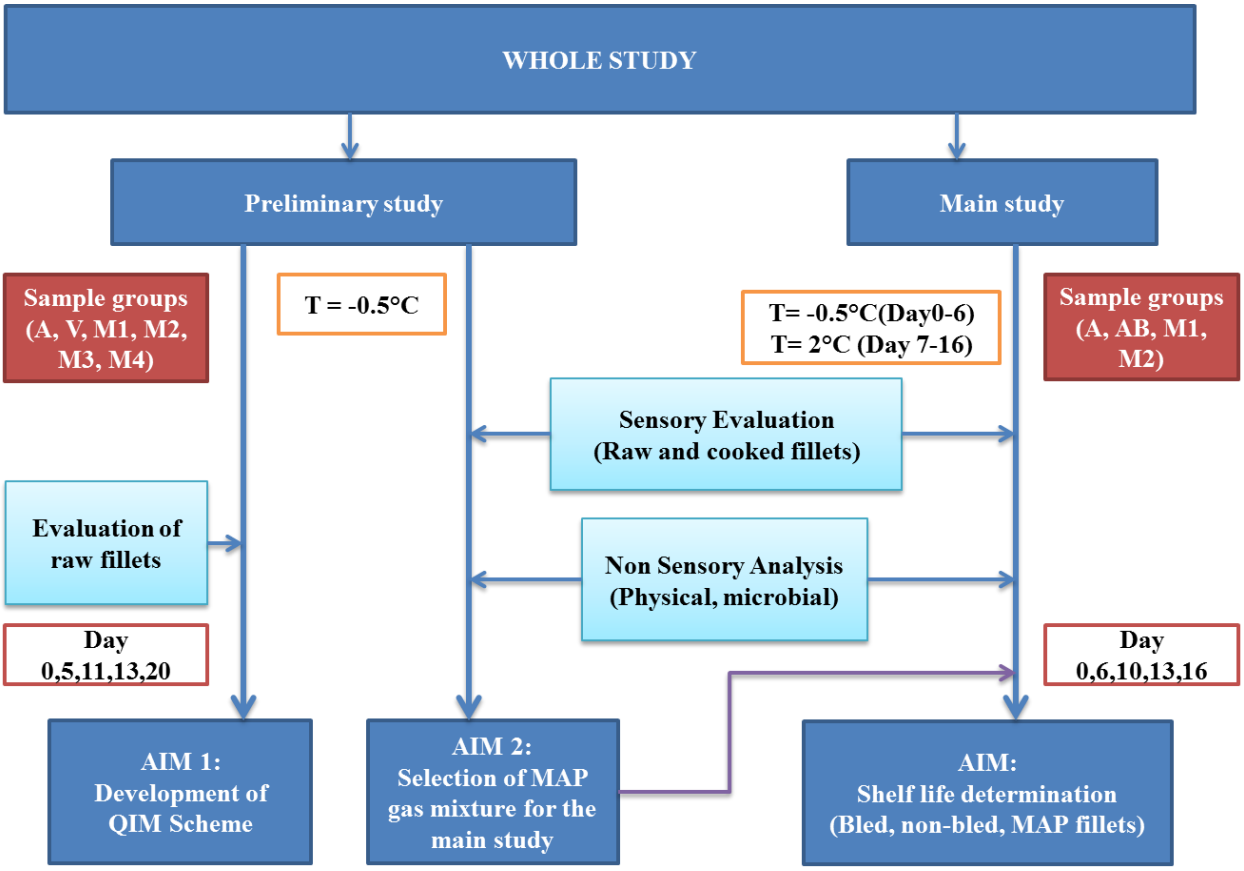


Figure 2: Chart showing experimental design for the entire redfish fillets study. Effects of gas mixtures (defined in table 3 and 4), storage temperature, drip loss and microbial growth on quality of the fillets were assessed.

The main study aimed at determining the effect of gas composition in the M1 (40% CO_2: 60% Ar) and M2 (40% CO_2: 60%N_2) identified during the preliminary study and bleeding of fish (AB) on the sensory quality and shelf life of redfish fillets during a shelf life study. The effects were compared to a control sample of non-bled and air packaged fillets in air (A). Samples were stored superchilled at -0.5°C to -1°C from day 0 to 6 and then chilled at 2°C from day 7 to end of storage (day 16). All the analyses were was carried out at regular intervals (on day 0, 6, 10, 13 and 16) throughout the study. Drip loss of the fillets from every sampled tray was determined on day 6, 10, 13 and 16 while evaluation of raw fillets (n=5 per sample group), headspace gas composition from every sampled trays and microbial analysis were analysed on each sampling day. Sensory evaluation of the cooked fillets (n= 12 per sample group) using Torry and Quantitative Descriptive Analysis (QDA) schemes was carried out up to day 16 of the study.

3.2 Preliminary study

3.2.1 Packaging

Whole redfish caught on 6/12/12 were processed (filleted and deskinned) one day post catch at HB Grandi, a fish processing factory in Reykjavik, Iceland. A total 35 kg of fresh fillets were packaged in 5 kg styrfoam (insulated) boxes with absorbent drip pads, covered with plastic sheet and ice mats for cooling. The fillets were transported to Matis (Icelandic Food and Biotech R&D) laboratory. On arrival at Matis, fillets of varying weights and sizes were laid on polystyrene trays, six fillets per tray and inserted into polyethylene (PE) film bags prior to flushing with different modified atmosphere gas treatments (Figure 3). Approximate weight of the polystyrene trays and the packaging film (PE bags) was determined before packaging. Temperature logger was placed underneath the fillets in an identified tray from each group.

Figure 3: Deskinned redfish loins (left), loins laid in tray (middle) and trays in film bags ready for modified atmosphere packaging.

Table 3 shows the packaging atmosphere conditions for each sample group. Prior to modified atmosphere packaging, gas composition for sample groups M1, M2, M3 and M4 was set. Pre-trial packaging using gas mixer MAP Mix 9000 (PBI-Dansensor, Ringsted, Denmark) was done to ensure correct gas mixture for each sample group. Head space gas composition was analysed using oxygen and carbon dioxide analyser (Checkmate 9900 Analyser, PBI-Dansensor, Ringsted, Denmark). Air stored samples (A) were not sealed to mimic air storage conditions in the Styrofoam boxes. All the packaged fillets were stored in a chamber at -0.5°C to 0°C for 20 days.

Table 3: Definition of packaging atmosphere conditions in sample groups.

Sample Group Code	Modified Atmosphere Treatment			No of Packages (trays)
	CO_2%	Ar%	N_2%	
M1	40	60	-	6
M2	40	-	60	6
M3	60	40	-	6
M4	60	30	10	6
V	Vacuum Packaging			6
A	Air storage			6

3.2.2 Development of QIM scheme.

The QIM scheme was developed following the 3 steps of QIM scheme development according to the methodology earlier described by Martinsdóttir *et al.* (2001) and Hyldig *et al.* (2007). The initial assessment was aimed at identifying the sensory descriptors to be monitored during storage time in the development of the draft QIM. Developed QIM for cod fillets (Cardenas Bonilla *et al.* 2007) was carefully studied before the evaluation. Two panellists evaluated and compared 6 fresh raw fillets (filleted one day post-harvest) and 6 old fillets (11 days old, stored at 0°C) on the first day (day 0) of the study. Colour of the fillet on the backbone and skin side, gaping, texture, odour and appearance were noted as main parameters that changed clearly with storage time. Changes in the parameters were subsequently assessed on day 5, 11, 13 and 20. During each sampling, 6 fillets from each sample group were assessed. Furthermore, photographs were taken in each sensory evaluation session as references for visual sensory attributes changes during storage. All observations of fillets were conducted under standardized conditions at room temperature. Each descriptor was associated with a demerit point ranging from 0 to 3, in which 0 corresponded to the descriptor denoting very fresh fillets. The highest scores for all the quality descriptors were summed up to give the overall Quality Index (QI).

3.2.3 Sensory evaluation of cooked fillets (Torry scheme)

Sensory evaluation of the cooked fillets was done using the Torry scheme for medium fat fish as originally described by (Shewan *et al.* 1953) with some modifications. A team of 12 panellists, trained according to international standards (ISO 1993), including detection and recognition of tastes and odours, training in the use of scales, and in the development and use of descriptors, evaluated cooked fish fillets using Torry scheme on day 0, 5, 11 and 13 of sampling. Samples weighing about 40 –50 g were taken from the fillet loins and placed in aluminium boxes coded with three- digit random numbers. The samples were cooked at 95-100°C for 6 minutes in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) with air circulation and steam, and then served to the panellists. Texture, odour and flavour were rated on a scale from 10 (very fresh fillets) to 3 (spoiled fillets).

3.3 Main study

3.3.1 Packaging

Whole redfish trawled on 12/01/13 were processed (filleted and deskinned) two day post catch at HB Grandi, a fish processing factory in Reykjavik, Iceland. A part of the fish were bled at sea for 10 minutes, the other part was not bled as is usually done on-board Icelandic trawlers. A total of 40 kg of unbled and 10 kg of bled fillets were packaged in 5 kg Styrofoam (insulated) boxes with absorbent drip pads, covered with plastic sheet and ice mats for cooling. The fillets were transported to Matis laboratory. At Matis, fillets of varying weights and sizes were laid on polystyrene trays, six (6) fillets per tray and inserted into polyethylene (PE) film bags prior to flushing with different modified atmosphere gas treatments (Table 4) using gas mixer MAP Mix 9000 (PBI-Dansensor, Ringsted, Denmark). Approximate weight of the polystyrene trays and the packaging film (PE bags) was determined before packaging. Temperature loggers were placed in some identified trays from each sample group. All the packaged fillets were superchilled at -0.5 to -1°C in a chamber until day 6 of the storage study and then chilled at 2°C from day 7 to end of storage study (day 16).

Table 4: Definition of packaging atmosphere conditions in sample groups.

Sample Group	Package Treatment	Number of packages
A	100% air and not sealed*	13
AB	100% air and not sealed*	13
M1	MAP- 40% CO_2: 60% Ar[a]	21
M2	MAP- 40% CO_2: 60% NO_2[a]	21

*Trays were inserted into PE film bags.

[a] Trays were inserted into Vacuum Packaging bags. Procedure for gas flushing is as explained in the preliminary study MAP for the sample groups.

3.3.2 Training of sensory panel and evaluation of raw fillets (QIM)

The 2nd step in QIM development involved training of panellists and finalizing the preliminary QIM scheme. Twelve panellists from Matis (Icelandic Food and Biotech R&D) laboratory were identified and trained during 3 training sessions. Ten panellists were familiar with raw fish freshness evaluation using QIM schemes for other fish species while two had not used QIM scheme before. The preliminary scheme was explained to the panellists during the 1st and the 2nd training sessions. Panellists were made aware of the storage time and conditions as they discussed and familiarized with red fish fillets of different stages of freshness. Redundant descriptors identified by the panel members were removed from the preliminary QIM scheme and new suggestions included to further improve the scheme. The third session involved evaluation of blind coded samples without prior knowledge of storage time and condition until after the evaluation. In the final step of QIM scheme development, final scheme was evaluated in the shelf life study of air packaged (bled and un-bled) and MAP (M1 and M2) redfish fillets stored in two different storage temperature conditions. The fillets were first stored superchilled at -0.5°C to -1°C until day 6 of the study and stored at 2°C until the end of storage (day16) Eight to twelve trained panellists evaluated raw fillets on fixed sampling days, 0, 6, 10, 13, 16, Fillets (n=5) from each sample group (A, AB, M1 and M2), coded with random three digit numbers, were presented in a random order and evaluated individually in 2 sessions. QI for all the replicates (n=5), in each sample group was calculated. Average QI for the replicates (n=5) given by individual panellists (n=12) in each sampling day was also determined.

3.3.3 Evaluation of dark and red and red spots on the fillets

During the preliminary study, the evaluated fillets were observed to have presence of dark and red spot patches that had negative impact on the appearance of the fillet. In the main study, this characteristic was rated on a 5 points scale (Table 5.) Fillets with less than 10% dark and red spots scored 0 while those with more than 80% of the spots scored 5 points.

Table 5: Scoring scale for red and dark spot areas on the fillets.

Parameter	**Description**	**Score**
Red or dark spot areas on the loin	Less than 10% of the loin	0
	10-20% of the loin	1
	20-40 % of the loin	2
	40-60% of the loin	3
	60-80% of the loin	4
	More than 80% of the loin	5

3.3.4 Sensory evaluation of cooked fillets

Parallel to evaluation of the raw fillets using QIM scheme, attributes of cooked fillets were evaluated by 8-12 trained panellists in two sessions during each sampling day using Torry scheme and QDA method. Panellists from Matis sensory panel familiar with the QDA evaluation method were trained in three sessions according to International Standards (ISO 1994) The cooked samples were also evaluated using modified Torry freshness scale for medium fat fish (Shewan *et al.* 1953). Samples weighing 40–50g were taken from the loin part of the fillets 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, Eglfing, Germany) with air circulation and steam and served to the panellists. QDA sensory vocabulary was developed through an interactive process during the training and consisted of 30 defined attributes for odour, flavour, texture and appearance of the cooked fillets (Table 6). The intensity of attributes was described using an unstructured scale (from 0 to 100%). Each panellist evaluated duplicates of samples in a random order for each group during fixed sampling days; day 0, 6, 10, 13, and day 16 (Table 6). A computerized system (FIZZ, Version 2.0, 1994-200, Biosystemes, France) was used for recording and processing data.

Table 6: Sensory attributes for cooked redfish fillets and their definitions.

Sensory attribute	Short name	Definition
sweet odour	O-sweet	Sweet odour of fresh redfish
cod liver	O-liver	Boiled cod liver
shellfish, algae	O-shellfish	Characteristic, fresh odour
vanilla/warm milk	O-vanilla	Vanilla, sweet warm milk
boiled potatoes	O-potatoes	Whole, hot, boiled potatoes in a saucepan
rancid	O-rancid	Rancid odour
dish cloth	O-cloth	Dirty damp dish cloth from the kitchen (left for 36 hrs.)
TMA	O-TMA	TMA odour, reminds of dried salted fish, amine
queasy sweet	O-queasy	Spoilage odour, queasy sweet, overripe fruits
spoilage sour	O-sour	Spoilage sour, sour odour, sour milk, acetic acid
Sulphur	O-Sulphur	Sulphur, matchstick, boiled cabbage
colour	A-colour	Light: white colour. Dark: yellowish, brownish, grey
heterogeneous	A-heterog.	Homogenous: even colour. Heterogeneous: stains, uneven colour
white precipitation	A-precipit.	White precipitation on the sample surface
flakiness	A-flakes	The fish sample slides into flakes when pressed with a fork
cod liver	F-liver	Boiled cod liver
metallic	F-metallic	Characteristic metallic flavour of fresh redfish
sweet	F-sweet	Characteristic sweet flavour of fresh redfish
rancid	F-rancid	Rancid flavour
pungent	F-pungent	Pungent flavour
queasy sweet	F-queasy	Spoilage flavour queasy sweet, overripe fruits
sour	F-sour	Spoilage sour, sour taste,
TMA	F-TMA	TMA flavour, reminds of dried salted fish, amine
off-flavour	F-off	Intensity of off-flavour (spoilage flavour)
soft	T-soft	Softness in first bite
juicy	T-juicy	Dry: draws liquid from mouth. Juicy: releases liquid when chewed
tender	T-tender	Tenderness when chewed
mushy	T-mushy	Mushy, porridge like texture
meaty mouth feel	T-meaty	Reminds of meat texture, rough fibres
sticky	T-sticky	Glues together teeth when biting the fish.

3.4 Microbiological analysis

Sample fillets, from each sample group were taken to estimate total viable counts (TVC). 25g of minced fillets were each, were transferred aseptically into a stomacher bag containing 225ml of buffer solution and blended for 60 seconds to obtain a 10-fold dilution. Further decimal dilutions were made. 0.1 ml of each dilution was pipetted onto the surface of iron agar plates in triplicates and spread on the plates. Enumeration of TVC was done after every 5 days of incubation at 17°C.

3.5 Physicochemical analysis

3.5.1 Headspace gas Composition

Headspace gas composition inside the modified atmosphere packages in the preliminary and the main study was analysed before opening the packages during each sampling using oxygen and carbon dioxide analyser (Checkmate 9900 Analyser, PBI-Dansensor, Ringsted, Denmark). A septum was placed on the package and gas aliquot was withdrawn twice for analysis of relative oxygen and carbon dioxide content in the MAP packages.

3.5.2 Drip loss in the packages

Drip loss in the preliminary and the main study was determined based on weight of the fillets in the package, weight of the empty tray before packaging and the weight of the wet tray during sampling. Drip loss % was calculated as the ratio of water lost during storage to the initial weight of the fillets × 100%.

3.5.3 Temperature monitoring

Automatic temperature data loggers (Stow Away®, Onset Computer Corp., Bourne, Mass., U.S.A.) were used to monitor product core temperature and ambient temperature in the storage chamber throughout storage time in the preliminary and the main study. Loggers n=1 in the preliminary study and n=3 in the main study, were placed underneath the fillets in the selected trays from each sample group. Ambient temperature of the storage chamber was monitored by placing the loggers (n=4) at different points within the chamber in both studies. Temperatures were read at the end of the preliminary and main study. Temperature readings were made at 10 minutes intervals.

3.6 Data Analysis

Microsoft Excel 2010 was used for data processing, to calculate means and standard deviations for all multiple measurements and to generate graphs. Analysis of variance (one-way ANOVA) was performed on sensory, microbiological and physico-chemical data in the statistical program NCSS 2000 (NCSS, Utah, and USA). Comparisons of treatments were done using the Duncan's Multiple-Comparison Test (sensory data), Tukey-Kramer Multiple-Comparison Test (microbiological and physico-chemical data) and Kruskal-Wallis Multiple-Comparison Z-value Test (for non-parametric independent group comparisons) with a threshold level for significance of 5%.

4 RESULTS

4.1 Preliminary study

4.1.1 Development of Quality Index Method (QIM) for redfish fillets

Initial evaluation of redfish fillets by comparing the sensory attributes of the fresh and old fillets was aimed at identifying individual visible parameters that were noticeably different between the two sample groups. Colour of the fillet on the backbone and skin side, gaping, texture, odour and appearance were noted as main observable parameters that changed clearly with storage time. Subsequent observation of each parameter of the superchilled fresh fillets in air, vacuum packaged and MAP until spoilage resulted to a detailed description of sensory changes that were structured into a preliminary QIM scheme. During the training sessions, suggestions for improvement of the scheme resulted in a final QIM scheme. Appearance and odour were modified using descriptors more suitable for the parameters. All the parameters noted were found to be relevant in the freshness evaluation of redfish fillets. Table 7 shows the final QIM scheme with sum QI score of 16 points.

Table 7: Final QIM Scheme developed for deskinned redfish fillets.

Quality Parameters		**Description**	**Score**
Colour	backbone side	Bluish , transparent	0
		Slightly milky	1
		Milky	2
		Yellowish, brown spots toward the tail	3
	Skin side	Dark muscle bright, reddish brown	0
		Dark muscle less bright, brown	1
		Dark muscle brown, yellowish	2
Gaping		No gaping, one gaping in the thick loin	0
		Slight gaping, flesh torn less than 25 %	1
		More gaping, flesh torn 50-75 %	2
		Deep gaping , flesh more torn	3
Appearance		Glossy, bright	0
		Not glossy, slightly waxy	1
		Waxy	2
Texture		Firm	0
		Fairly firm	1
		Soft	2
		Very soft	3
Odour		Fresh, marine	0
		Neutral, cucumber	1
		Sour, hay, melon	2
		Off odour, putrid	3
Quality Index(0-16)			**SUM**

4.1.2 Selection of MAP gas mixture for the main study

Selection of the MAP gas mixture was based on the effect of different packaging methods on the sensory quality of superchilled redfish fillets in relation to headspace gas concentration and drip loss

4.1.2.1 Concentration of CO_2 and O_2 in the headspace

Initial headspace gas composition (day 0) in the packages aimed to contain gas mixtures for the MAP sample groups as defined in Table 3 while Figure 4 shows changes in headspace gas composition during storage. Equilibrium normally occurs between 12 and 48 hours after packaging (Lauzon *et al.* 2011). Decline in the level of headspace CO_2 was observed between day 0 and 5 in all MAP groups. However, the concentration of CO_2 after day 5 remained relatively constant until day 11 when the final gas measurements were determined. The level of O_2 on the other hand increased until day 5 but remained relatively stable up to day 11.

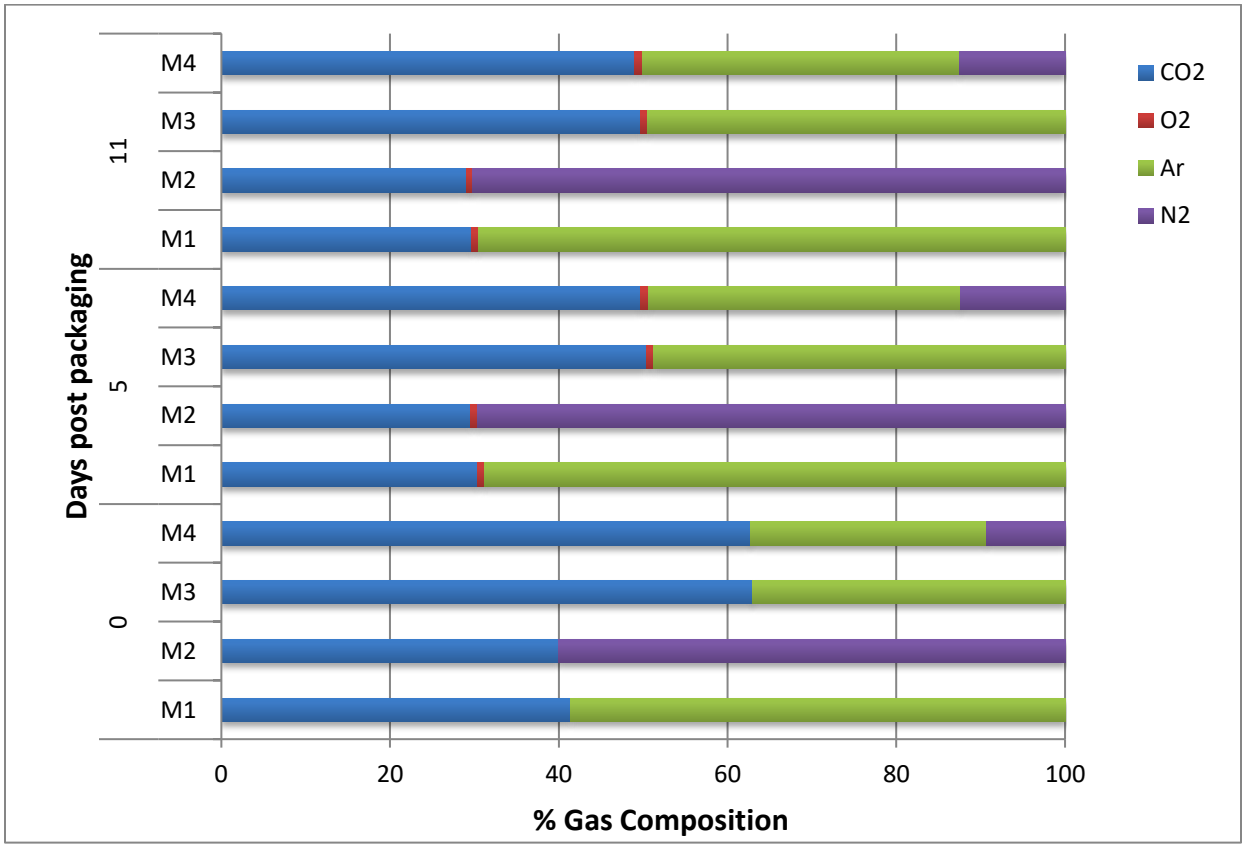


Figure 4: Mean headspace gas composition (% CO_2, O_2, Ar, N_2) in trays (n=3) sampled during storage (day 0, 5, 11).

4.1.2.2 Drip loss %

Table 8 shows average drip losses with standard deviations for all the sample groups. Drip loss was determined on day 6 and day 11 of storage. Day 0 was assumed to have 0% drip loss. Higher drip loss on day 5 was observed in the V (4.25±0.70) and M4 (4.04 ± 0.15) samples compared to the other sample groups while on day 11, highest drip loss was observed in M3 and M4 MAP groups with no significance difference between them.

Table 8: Average drip loss % (n=3) of the sample groups (A,V,M1,M2,M3,M4) and Standard deviations (% ± SD).

Day	A	V	M1	M2	M3	M4
5	1.51(0.50)	4.25(0.70)	2.70(0.48)	2.93(0.48)	3.92(0.55)	4.04(0.15)
11	2.11(0.48)	5.28(0.46)	6.10(0.59)	4.96(1.15)	7.38(2.20)	7.13(0.53)

4.1.2.3 Temperature monitoring (preliminary study)

Mean temperature of the products and the ambient temperature of the chamber are shown in Figure 5. Temperature profile of the product and storage chamber was monitored during the 20 day period of the study. Initial mean product temperature for all the sample groups (A, V, M1, M2, M3 and M4) was 2.7 ± 0.5°C while the mean product temperature during storage (20 days) was -0.3 ± 0.3°C. Loggers (n=4) placed at different position in the chamber gave a mean ambient temperature of -0.4 ± 0.6°C during storage (20 days). Temperature was stable except during sampling days. However, higher temperature on the 8th day could not be explained.

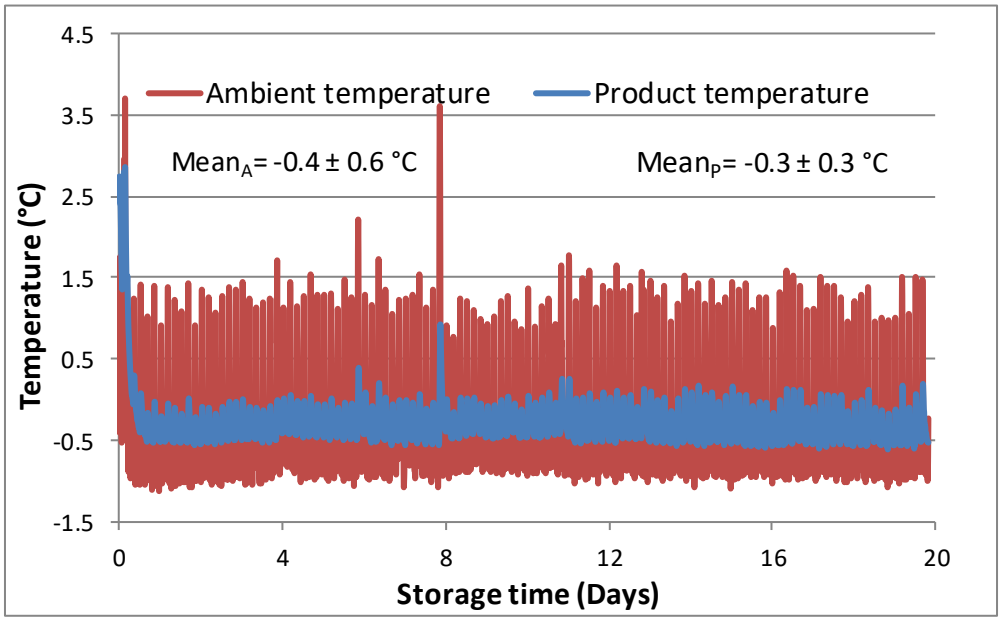


Figure 5: Mean ambient temperature of the chamber (Mean $_A$) and product temperature (Mean $_P$) of the sample groups during 20 days storage in the preliminary study.

4.1.2.4 Sensory evaluation of raw and cooked fillets (Torry)

Evaluation of raw fish fillets showed evolution of sensory changes in parameters until spoilage. Colour, texture, odour, gaping and appearance were monitored until day 20 of storage. The degree of evolution in each parameter was observed to be influenced by the packaging condition of the samples (A, V, MAP). Very fresh fillets were considered to be glossy and bright with characteristic bluish and transparent colour, fresh marine odour and intact muscles with firm texture. However, the parameters evolved at different rates within the sample groups until spoilage to give waxy appearance, yellowish brown colour, and putrid odour and, soft and torn muscles. Spoilage odour (off odour) was perceptible in A and V samples on day 11 and not detected in the MAP samples throughout storage (day 20). Texture of the thick loin was fairly firm in the MAP samples until day 13 except in M4 where it was noted to be inferior (soft) on day 11. Gaping was evident in all the sample groups by day 5 of the study and the extent progressively varied until day 20 of storage. Sensory evaluation of cooked samples using Torry scheme was used as reference for the raw fish evaluation. Eating quality for all the sample groups decreased with storage time shown in Figure 6. Torry score of 7 indicates end of freshness while 5.5 (shown by the red line) is the sensory rejection score due to detection of spoilage attributes. V reached consumption limit on day 9 while A, M3 and M4 were rejected on day 11. M2 showed better than M1 until day 11.

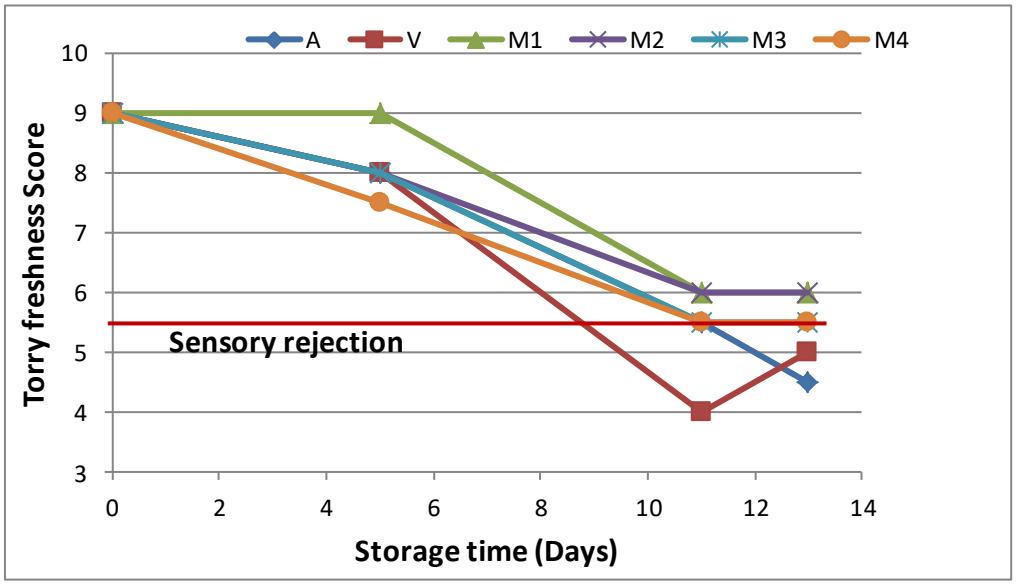


Figure 6 : Mean Torry freshness scores for cooked redfish loins of the sample groups(A, V, M1, M2, M3, M4)during storage. Torry score 5.5 shows the sensory rejection point.

4.2 Main study

4.2.1 Sensory evaluation of raw fillets using QIM

Figure 7 represents panellist's performance based on average QI for sample group A on day 0,6,10 and 13. The results show that the fillets received increasing QI scores with storage time by most of the panellists. Average QI for Panellists P2 was observed to be high on day 6 and declined progressively at 10 and 13. Panellist 4 scored higher at day 0 and then low at day 10. Similarly initial QI score for panellist P6 was high at day 0, low at day 10 and later high at day 13. Similar

performances for panellists 2, 4, and 6 were observed for sample group AB, M1 and M2 (figures not shown in this report).

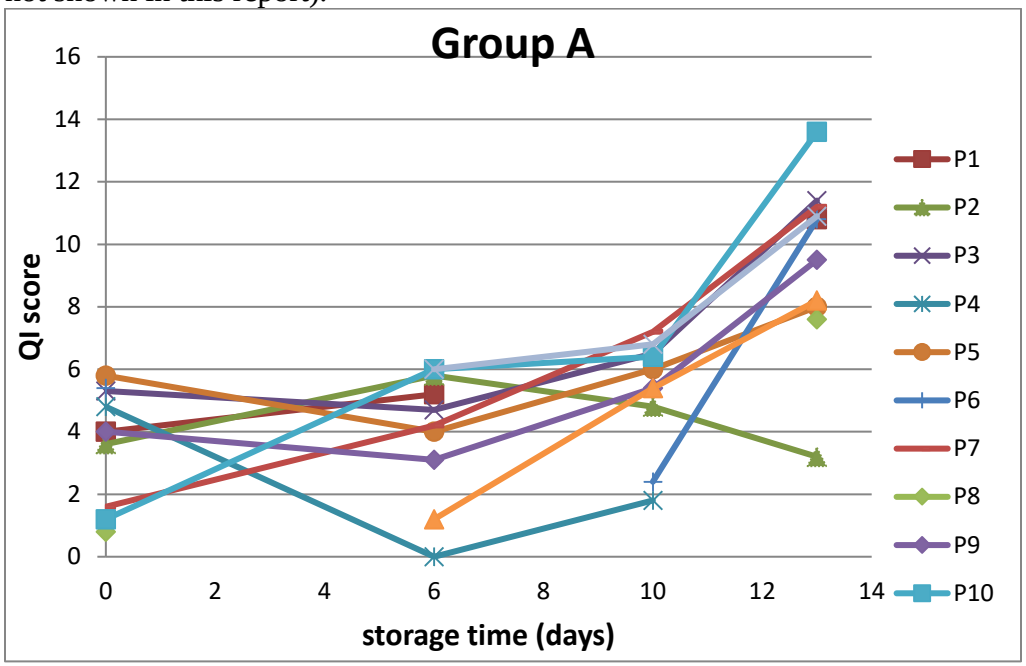


Figure 7: Performance of the panellists based on the QI score for sample group A during each sampling day in the shelf life study versus storage time.

Due to the inconsistency in scoring compared to the other panellists, data from panellist 2, 4 and 6 was excluded from QIM analysis and strong linear correlation (R^2 = 0.8464) between average QI and storage time was obtained, as represented by the linear equation in Figure 8.

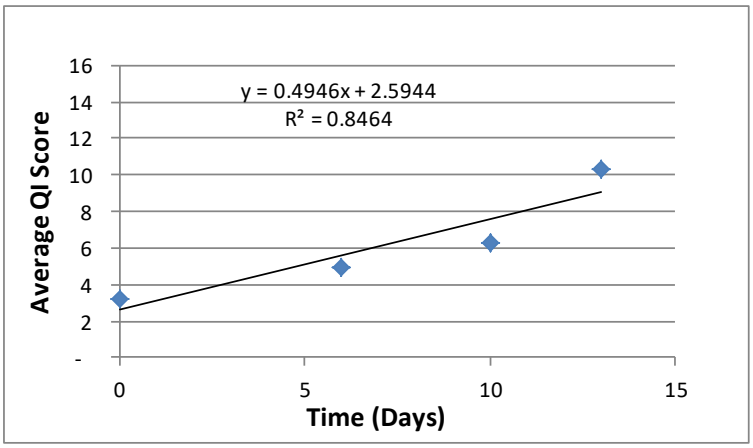


Figure 8: Linear correlation of average QI of the panellists per sampling day and storage time.

Panellists (n=9) evaluated replicate samples (n=5) for all the sample groups. Figure 9 shows average QI of the replicates (n=5) for all the sample groups from the panellists during storage. Great variation in QI of the replicates was observed in all sample groups in each sampling day.

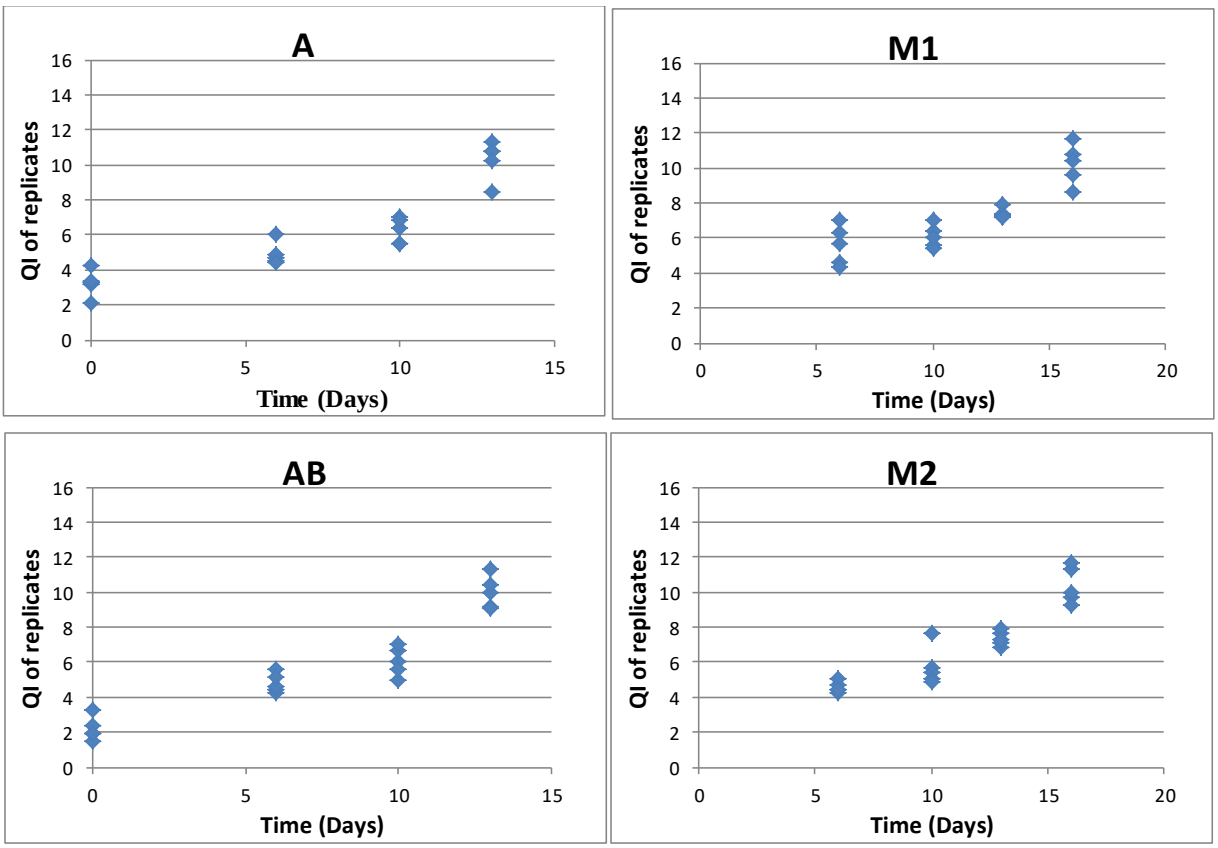


Figure 9: QI of the replicates (n=5) for all the sample groups during storage. 9 panellists evaluated the samples.

Six attributes (colour of skin side and the backbone side, appearance, odour, gaping and texture) were evaluated using the QIM scheme. Average QI for the sample groups was based on evaluation of replicates (n=5) by the panellists. In all the samples, the QI was linearly related to storage time (Figure 10) with high correlation between the average QI and storage time; A (R^2=0.8464), AB (R^2= 0.9097), M1 (R^2=0.9155) and M2 (R^2=0.888). Similar average QI score of 10 was obtained during the final sampling; day 13 for A and AB and day 16 for M1 and M2 samples.

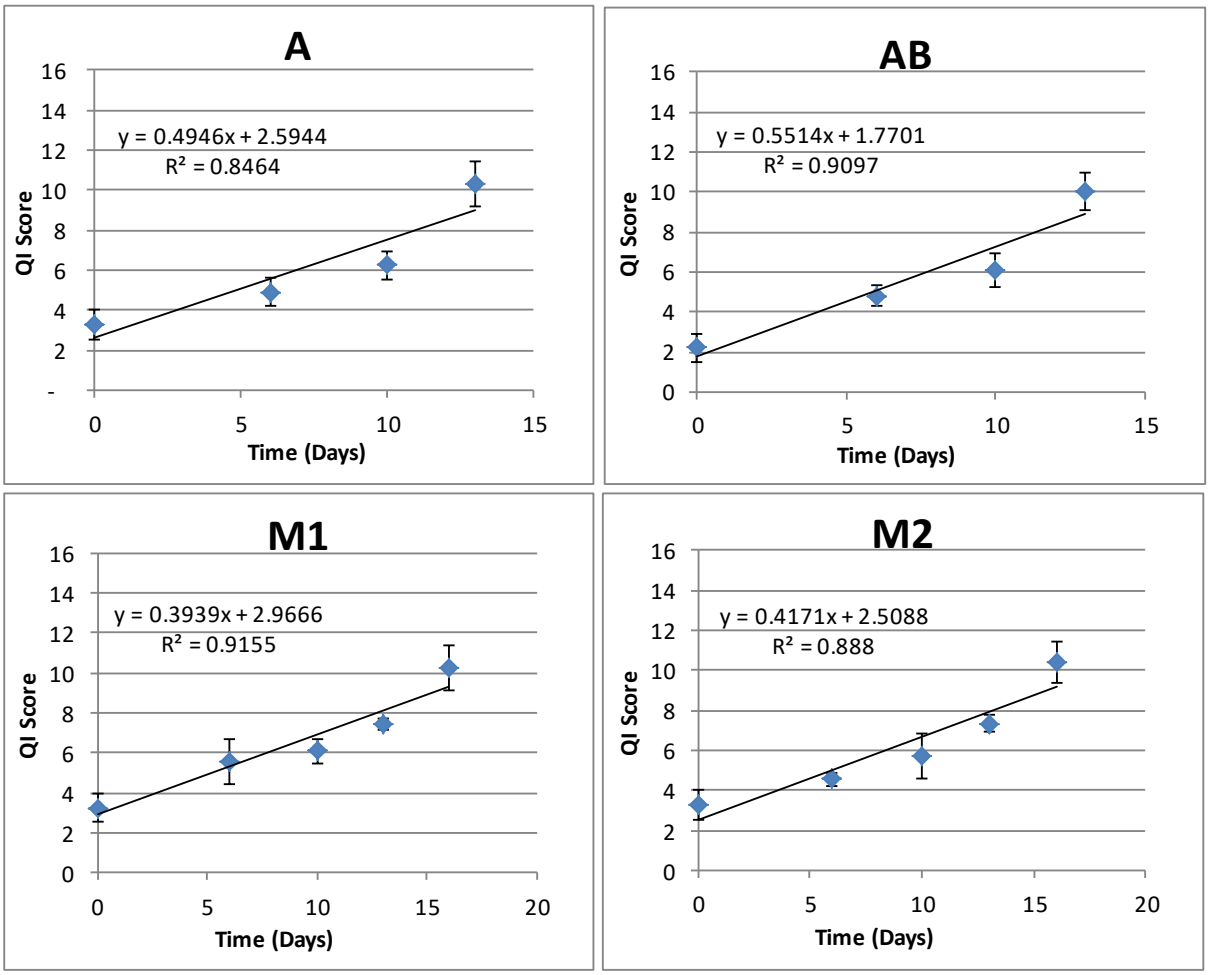


Figure 10: Average QI scores and standard deviation (± SD) for sample groups A, AB, M1, and M2 based on replicate evaluation of the samples (n=9) by 9 panellists during storage.

Attributes of the raw fillets were observed to increase with storage time (Figure 11). However variations between the groups were evident on different sampling days. Scores for colour (backbone and skin side), appearance and odour attributes changed sharply with storage time compared to gaping and texture that were rather gradual. All the attributes scored between 0 and 1 at the beginning of storage time. However, AB (bled fillets) scored lower in colour of the skin side, appearance and odour compared to A, M1 and M2 (non-bled fillets) at the beginning of storage time. During storage, minor differences between sample groups were observed in texture firmness and gaping. However, differences were observed in colour of skin and back bone side, appearance and odour between sample groups on day 13. By that time, A and AB scores were higher than of M1 and M2.

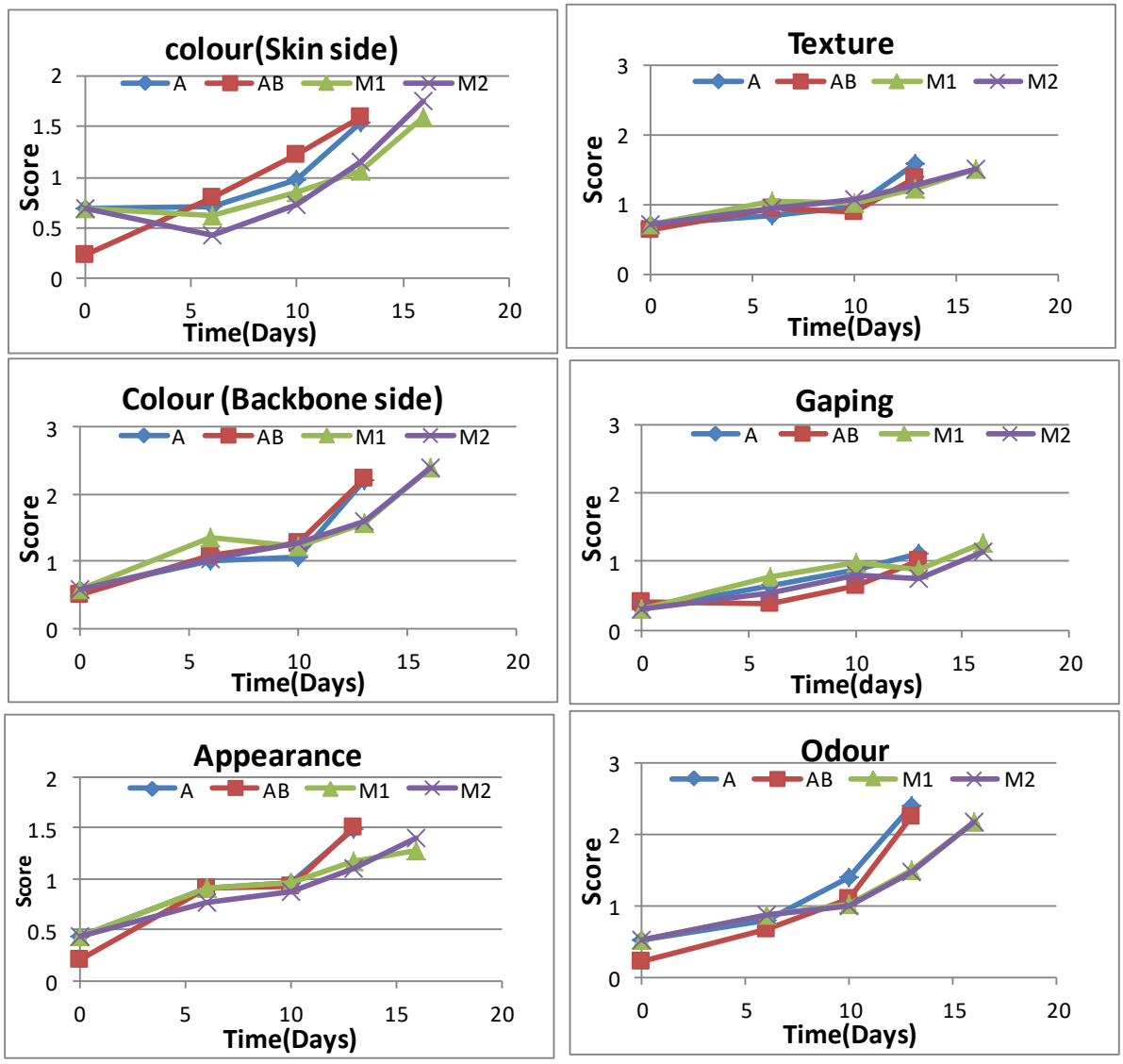


Figure 11: average score for individual quality attributes of superchilled (at -1˚C from day 0 to day 6 and chilled at 2˚C from day 6 to day16) fillets (n=5) evaluated in A, AB, M1 and M2 sample groups using QIM scheme for redfish fillets against storage time.

4.2.2 Evaluation of dark and red and red spots on the fillets

The initial score was between 0 and 1 in A and AB sample groups. However, different packaging methods and storage temperature did not influence changes in the parameter. The attribute scored between 0 and 1 throughout storage time (Figure 12). General appearance in AB (bled) fillets was better than in the other groups (non-bled).

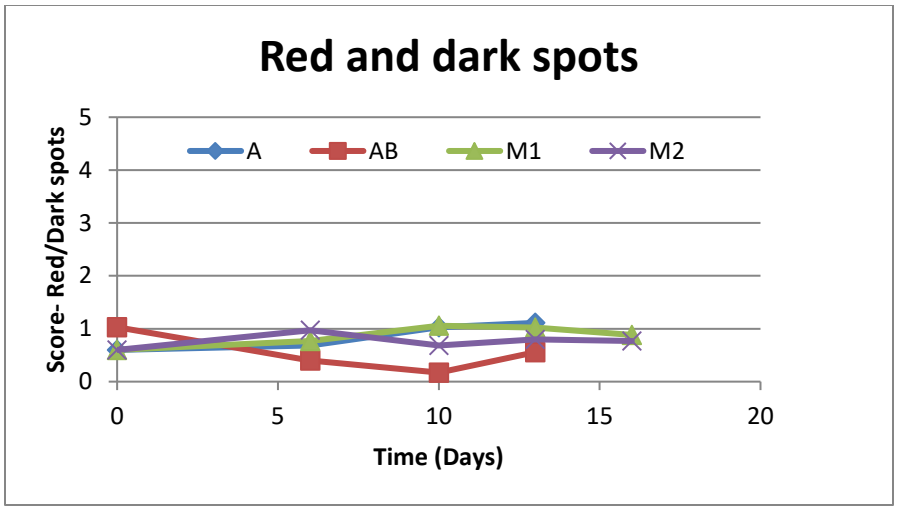


Figure 12: Evaluation of red and dark spot areas on the fillets with storage time.

4.2.3 Sensory evaluation of cooked fillets (Torry and QDA)

Results of the sensory evaluation of the cooked fillets using Torry during storage are shown in Figure 13. The Torry score decreased consistently in A and AB samples until day 6 to attain a Torry scale of 6.9 and 7.2 respectively. Freshness was rather constant after day 6 in M1 and M2 samples while in A and AB, gradual freshness decline was observed until day 10. A and AB reached sensory rejection threshold (Torry score 5.5) at day 11 and day 13 and 14 in M1 and M2 respectively.

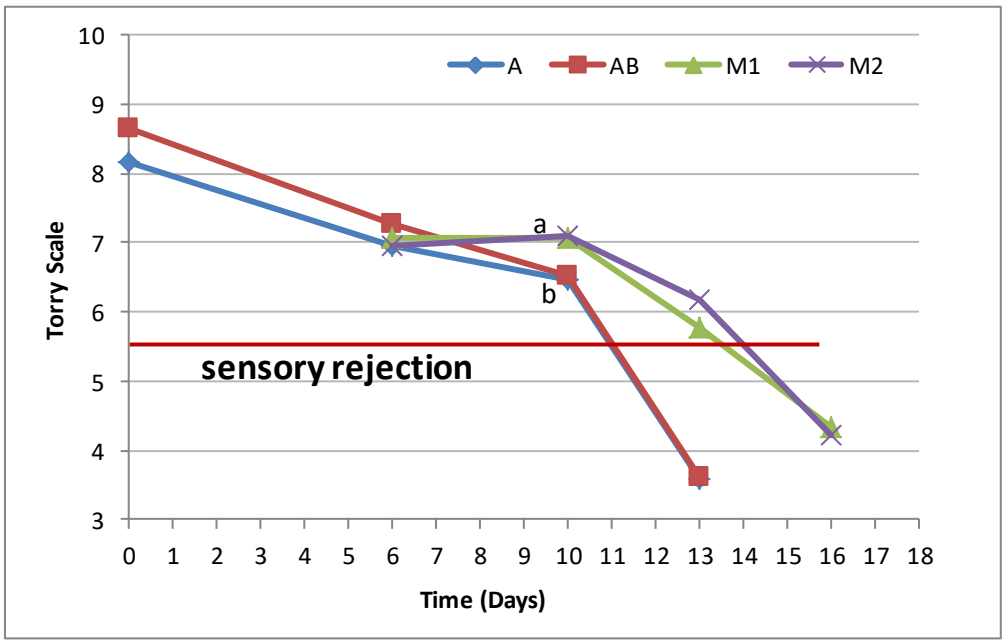


Figure 13: Mean Torry scores for A, AB, M1 and M2 during storage at superchilled (-1°C) from at day 0 to day 6 and then at (2°C) at from day 6 to day16.

QDA showed prominence of fresh odour and flavour attributes (liver, metallic, sweet, shellfish and vanilla) at the beginning of storage but the intensity reduced as storage time progressed.

Whereas rancid, cloth, TMA, queasy and off odour attributes became important as storage time progressed indicating spoilage (Figure 14 and 15). Differences between sample groups were noted by day 13, when spoilage related attributes (cloth, sour, TMA and queasy odours, sour flavour) became more noticeable in groups A and AB than in groups M1 and M2. Mean sensory scores (0-100) of the attributes for cooked redfish and level of significance are shown in Appendix 1 and 2.

4.2.4 Microbiological analysis

Changes in the value of the TVC in the sample groups (A, AB, M1, and M2) are represented in figure16. The initial TVC count in AB (3.3 log CFU/g) was higher than in A (2.6 log CFU/g). On day 6 TVC of the air packaged bled (AB) and unbled fillets (A) was observed to be higher than in the MAP fillets (M1 and M2). After day 6, TVC increased rapidly until end of storage; day 13 for A and AB and day 16 for M1 and M2 groups. Temperature in the storage chamber was adjusted on day 6 from -1°C to 2°C. Up to day 10, no difference in in TVC was observed in the M1 and M2 groups. TVC exceeded 7 log CFU/g which is considered as the maximum limit of acceptability on day 10 for A and AB samples and around day 16 for M1 and M2 samples. During the entire study microbial load in AB was found to be higher than in the other sample groups.

4.2.5 Temperature profiles during storage

The mean temperature of the sample groups (A, AB, M1 and M2) and the ambient temperature of the storage chamber are represented in the figure 17. Initial mean product temperature and the standard deviation for the sample groups (n=3) was 2.2 ± 1.0°C (A), 3.5 ± 0.5°C (AB), 2.1 ±1.0°C (M1) and 1.6 ± 0°C (M2). The samples were superchilled (-0.5°C) from at day 0 to day 6 and then chilled at 2°C from day 7 to end of storage. Mean ambient and product temperature during superchilled storage (day 0-6) was -0.7±0.9 °C and – 0.5±0.7°C respectively and 1.7 ± 0.4 °C and 1.9 ± 0.1°C respectively during chilled storage. Product temperature was stable. Fluctuations in ambient temperature were due to opening of chamber on sampling days.

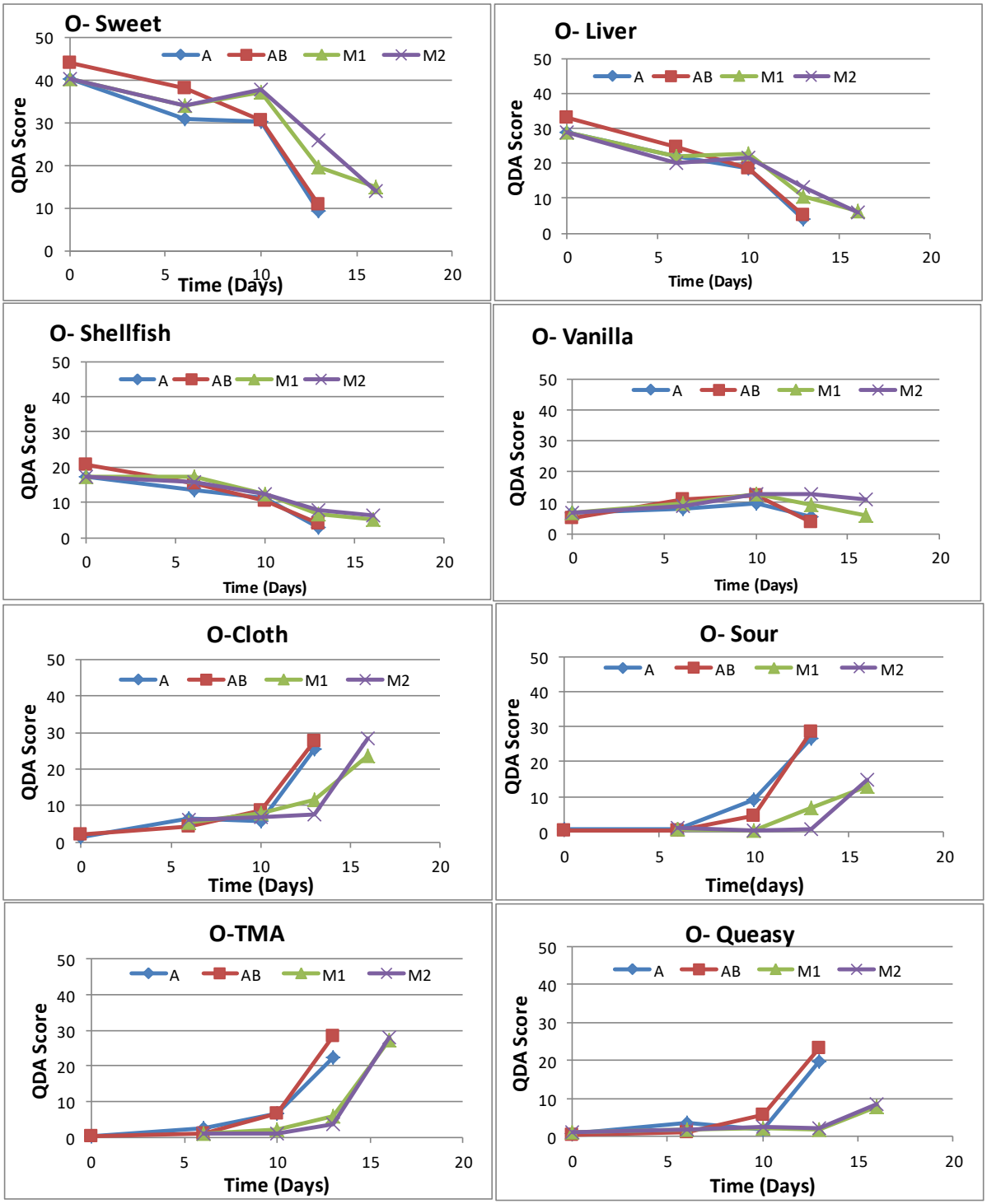


Figure 14: Average profile scores for positive (sweet, liver, shellfish, and vanilla) sensory and negative attributes (cloth, sour, TMA, queasy) in all sample groups (A, AB, M1, and M2).

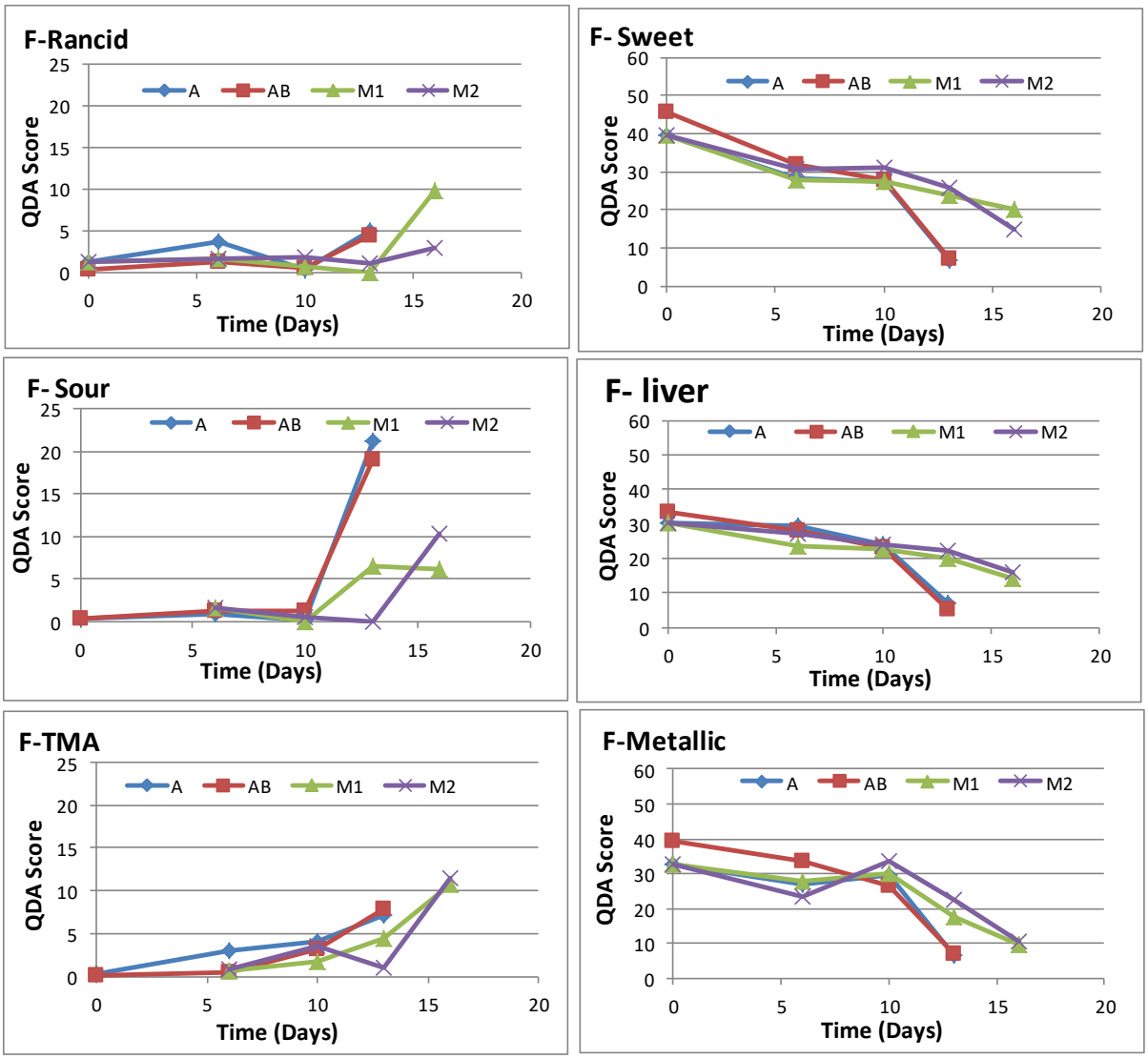


Figure 15: Average profile scores for negative (rancid, Sour, TMA) and positive (Sweet, Liver, and Metallic) flavour sensory attributes in all sample groups (A, AB, M1, and M2).

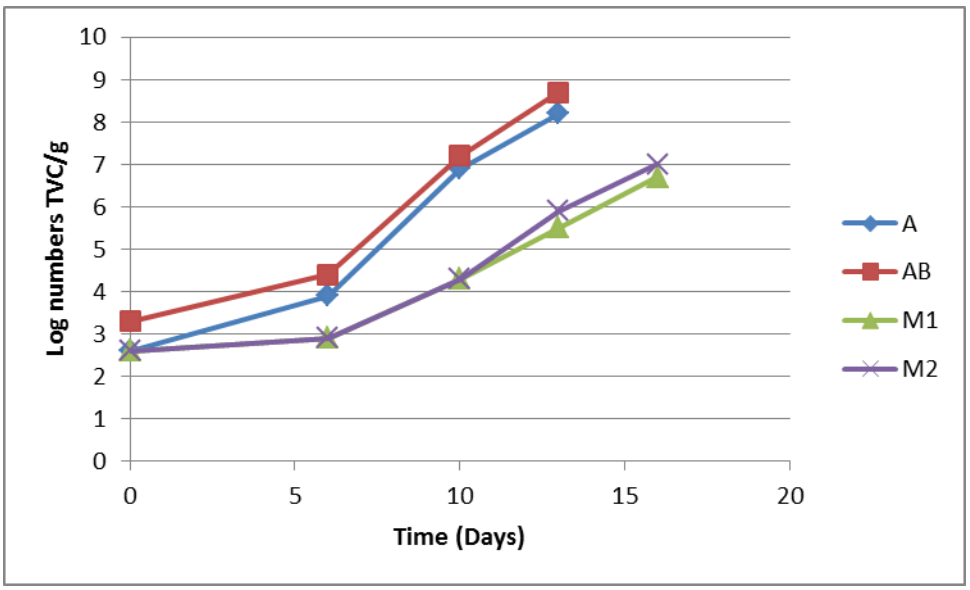


Figure 16: Total Viable Counts (TVC) and of sample groups (A, AB, M1, and M2) at superchilled (-1°C) from at day 0 to day 6 and then at (2°C) from day 6 to day16 of storage. Log numbers are in CFU/g.

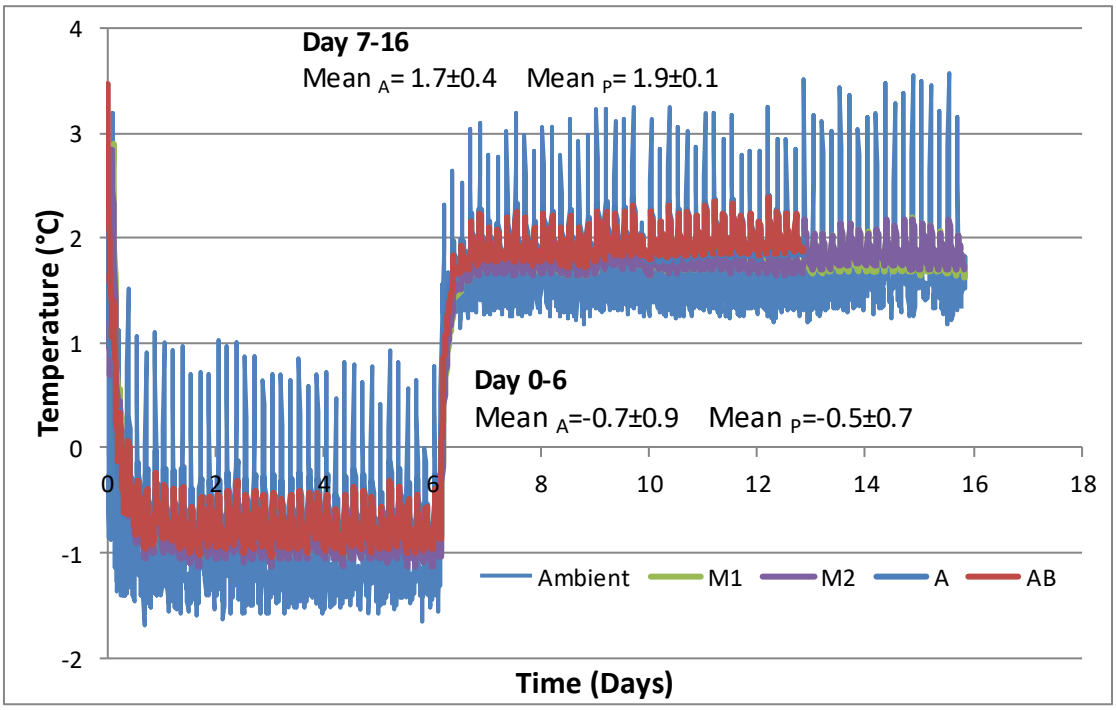


Figure 17: Mean temperature of the fillets in the sample groups (A, AB, M1, M2) and the ambient temperature of storage chamber during storage (16 days). Mean ambient (Mean $_A$) and product (Mean $_P$) during superchilled and chilled storage are shown.

4.2.6 Physical Analysis

4.2.6.1 Drip loss

Figure 18 shows drip loss % in the sample groups during storage. Day 0 was assumed to have 0% drip loss. M1 showed high exudate loss of 5.51% on day 10 compared to the other samples. On day 16 M1 and M2 showed the highest drip loss of 6.06% and 6.59% respectively. The entire experiment showed high exudate loss from the MAP samples (M1 and M2) compared to the air packaged sample (A and AB).

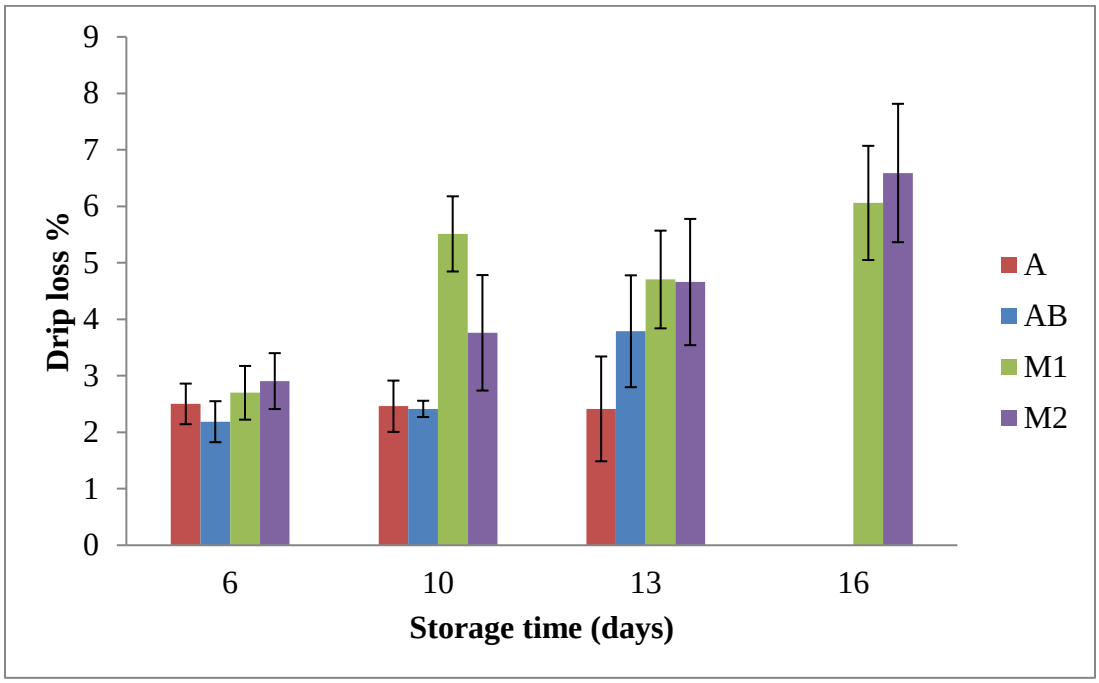


Figure 18: Average drip loss % of fillets (n=5) superchilled (-1°C) at day 0 to day 6 and then at 2°C from day 6 to day 16. A, AB, M1 and M2 sample groups were evaluated on during storage.

4.2.6.2 Headspace gas concentration.

Headspace O_2 and CO_2 levels in the MAP samples are shown in figure 20. The initial gas concentration of the samples was aimed at attaining headspace gas mixtures as defined on table 4. Similar to the observation made during the preliminary study, the level of CO_2 declined in the superchilled MAP packages between day 0 and day 6. However, the CO_2 concentration stabilized with increase in temperature to 2°C after day 6 of storage up to end of the study. The reduction was almost similar in M1 and M2 with no significance difference between the two treatments ($p > 0.05$). The level of O_2 in the headspace increased slightly to 1% on day 6 in both MAP groups (M1 and M2) and consequently stabilized up to the end of storage.

5 DISCUSSION

5.1 Development of QIM

A QIM scheme for deskinned redfish fillets was developed with a total sum of 16 QI points. Sensory attributes and their changes during storage time were described in a preliminary QIM scheme designed in a pre-study aimed at identifying sensory parameters of deskinned red fish fillets changing with storage time. All descriptors related to sensory attributes changing with storage were assigned demerit points according to the rate of freshness loss. Additionally, during training sessions before the shelf life study, the QIM scheme was slightly modified. This scheme was then used in a shelf life study to follow evolution of sensory changes of superchilled fillets packaged in air, vacuum and modified atmosphere during storage until spoilage (Table 7). Colour on the skin and backbone side, texture, odour, appearance and gaping attributed changed with storage time in the samples. In air and vacuum-packed samples, descriptors denoting spoilage were attained by the 11th day of the storage. MAP samples were further studied until day 20 of storage to attain the similar spoilage parameters as in A and V.

Colour description on the skin and the backbone side of the fillets was treated separately. On the skin side, dark muscle presented evolution of colour changes differently from the white muscles on the backbone side. Dark muscle contains higher levels of lipids and myoglobin than the white muscles (Huss 1995) therefore the discoloration is presumed to be as a result of their oxidation of during storage thus affecting the appearance of the fillets. More changes with storage time were noted in appearance of the skin side, resulting in a maximum of 3 demerit points, than on the skin side, which received a maximum of two demerit points.

Gaping was characterized by the degree of tearing or cracks in the fillet muscle. This was as a result of weakening of the connective tissue that holds the fish muscles together with storage time presumably due to action of autolytic enzymes (Huss 1995). The degree of gaping was compared to the whole fillet giving a maximum score of three demerit points. Evaluation of texture firmness was done in accordance to guidelines described by Martinsdóttir *et al.* (2001). Fresh fillets had initial firm texture but textural changes varied in the air, vacuum and MAP fillets with storage time. This may have been influenced by autolytic processes and drop in pH after dissolution of CO_2 into the fish muscle especially in the MAP samples(Huss 1995).

Glossy appearance of the fresh fillets progressively faded to waxy state at the end of storage time. Appearance was also negatively influenced by presence of dark and red patches on the fillet. The criterion did not evolve with storage time therefore not considered as freshness indicator. However, the criterion was evaluated in the main study using a separate rating scale.

Assessed fillets displayed individual variations. In the guidelines for freshness assessment of whole fish, Martinsdóttir *et al.* (2001) recommended use of at least three(large fish) and ten (small fish) samples to comprehensively study the spoilage changes. Sveinsdottir *et al.* (2002) also recommended use of at least three whole fish samples in order to increase precision in prediction of storage time. Studies by Cardenas Bonilla *et al.* (2007) and Wang *et al.* (2008) recommended use of at least three fillets due to their individual variations. In this study therefore, at least five fillets per sample group were recommended for a shelf life study.

5.2 Sensory evaluation of raw fillets using QIM for deskinned redfish fillets

In the shelf life study, results from the assessment of the panellists using the average QI scores for non-bled air packaged sample (A) clearly showed poor reproducibility of panellist 2, 4 and 6 based on the assumption that QI linearly increases with storage time (Figure 7) as explained by Martinsdóttir *et al.* (2001). A likely explanation for the poor performance of the three panellists could be linked to unfamiliarity with use of QIM and few training sessions conducted before its evaluation. A significant correlation (R^2= 0.8464) between the average QI of the panellists and the storage time was observed after excluding data from panellists 2, 4, and 6 in the QIM analysis thus showing linear QI increase with storage time (Figure 8). However, a stronger correlation (R^2=0.989) between average QI score of cod fillets and storage time in ice was shown by Cardenas Bonilla *et al.* (2007).The difference could have been as a result of few sampling points used in this study (four sampling points) and storage of the samples at different temperatures to simulate storage during transport by sea freight (storage at -1°C for 6 days) and then storage at retail (2 °C from day 6 until end of shelf life study).

Considering the QI score per replicate samples, some variation was observed in all groups (A, AB, M1 and M2) as shown in Figure 9. The variations in scores of individual fillets may have been contributed by differences in the individual fillets. Higher variation on day 13 was as a result of differences in sensitivity and understanding of attributes, but also different time of onset of the spoilage. Sveinsdottir *et al.* (2003) showed similar results in the usage of QIM for Atlantic salmon (*Salmon salar*) by the panellists. The panellist's performance therefore warranted further training to remove or minimize the variations by improving their awareness and understanding of the QIM (Tomic *et al.* 2007). In addition, use of at least 3 fillets per sample group would minimize the biological variations among the individual fillets

Strong linear relationship between the QI of the samples (R^2= 0.8464, 0.9097, 0.9155 and 0.8888 in A, AB, M1 and M2 respectively) and the storage time was observed (Figure 10). Attributes (Colour of the backbone and skin side, texture, gaping, odour and appearance) linearly evolved with variations among them (Figure 11). Changes on individual attributes varied among non-bled (A), bled (AB), M1 and M2 sample groups. Colour (backbone and skin side), appearance and odour attributes changed consistently with storage time while gaping and texture were observed to change rather gradually with storage time. Bleeding improved the initial colour of the skin side (white muscle) and the appearance compared to non-bled samples. However, the parameters changed sharply after superchilled (day 0-6) storage and in chilled storage (day 7-16). Bleeding was also found to improve initial odour (day 0) of air bled than non-bled fillets during storage. Similar results were shown by Maqsood and Benjakul (2011) in their study on effect of bleeding on lipid oxidation and quality changes of Asian seabass (*Lates calcarifer*).

Evolution of sensory changes in gaping and texture attributes was gradual and consistent in all the sample groups, reaching a maximum average score of 1.5 in texture and 1.2 in gaping. The highest score for both attributes in the QIM scheme was 3. More evolution of attributes was noted in the preliminary study than in main study resulting to higher demerit points. This was contributed by changes in air and vacuum samples and higher dissolution of CO_2 in M3 and M4 packages, in the 20 day storage of preliminary study. Texture remained relatively constant between day 6 and 10 in study. This could be associated with lower concentration of CO_2 in the MAP packages and increase in temperature that led to decline in dissolution of CO_2 in M1 and M2 samples in both

studies. Dissolution of CO_2 in the water phase of the fish forms carbonic acid. Low pH reduces water holding capacity of the proteins leading to soft texture (Wang *et al.* 2008). However degradation caused further softening of the muscles during storage.

Evaluation of dark and spot areas revealed inadequate information in relation to loss of freshness. The scale provided a range from 0 to 5 points (Table 5) but average score from the assessors ranged demonstrated use of a narrow part of the scale between 0 and therefore showing no direct relation to storage time (Figure 12). The scoring pattern could have been as a result of inadequate exposure to usage of the new scale due to few training sessions conducted before the shelf life study. More training in the use of the scale is would familiarize the panellists with the descriptors and improve their discrimination ability Labbe *et al.* (2004). Presence of dark and red spot areas was presumed to be as a result of poor fish handling before processing that caused rupture of fine blood vessels and consequent release of blood in the flesh with (Love 2001).This lowers the economic value of the fillets.

5.3 Sensory evaluation of cooked fillets using Torry scheme and QDA

In the preliminary study, evaluation of cooked fillets showed descriptors for absolute freshness on day 0 (Figure 6) and high Torry score of 9 in all the sample groups. M1(40% CO_2: 60% Ar) maintained freshness at Torry score 9 five days post packaging while the other sample groups showed decline in freshness. End of freshness characterized by Torry score 7 (Lauzon *et al*. 2011) was reached between day 7 and 9 of storage. M4 and V were observed to loose freshness at a higher rate while M1 maintained freshness longer than the other sample groups. At Torry score 7, freshness odour and flavour attributes are less detectable. Torry score of 5.5 has been used to determine end of shelf life, as when that score is reached, the samples become more characterized by presence of spoilage attributes (Martinsdóttir *et al.* 2001). The V sample group deteriorated faster than the other sample groups after day 5 and reached consumption limit at around day 9 while A, M3, and M4 were sensory rejected on day 11. M2 maintained better eating qualities throughout storage.

Statistical analysis of sensory evaluation of the cooked fillets using Torry scheme (Figure 13) in the shelf life study revealed no significance difference between all the sample groups (A, AB, M1 and M2) up to day 10 of storage. However, marginal significance existed between the groups on day 0 ($p = 0.060$) and day 10 ($p = 0.066$). On the first day, the bled group received slightly higher Torry freshness score as compared to non-bled. On day 10, the MA packed fillets received slightly higher Torry freshness score as compared to the air packed groups. Similarly, on day 13 the air and MA packed samples were different in Torry score ($p = 0.000$), but no significance difference existed between the two air stored samples (A and AB) or the two MAP samples (M1 and M2). Freshness declined with storage. Air packaged bled and non-bled samples reached consumption limit (Torry score 5.5) on day 11 while acceptability limit for M1 and M2 was around day 14.

According to the sensory evaluation using the QDA, fresh odour attributes (sweet, liver, shell fish, vanilla) remained relatively stable up to day 10 of storage for all the groups but became less evident towards the end of the storage time (Figure 14). On day 13, M1 and M2 had stronger sweet, liver, shellfish and vanilla odour than the A and AB groups. Sweet attribute appeared to be stronger compared to other fresh odour characteristics throughout the storage time. On the other hand, the spoilage attributes (cloth sour, queasy and TMA) became prominent with increasing storage time.

However, those changes occurred over different time periods for the groups. Significance difference ($p < 0.05$) in TMA and sour odour between the air packaged (A and AB) and MAP (M1 & M2) was observed on day 10.

Fresh flavour attributes (liver, metallic, sweet) were also prominent at the beginning of storage, and decreased with storage time in all the groups (Figure 15), while the flavour spoilage (rancid, pungent, queasy, sour, and off odour) became stronger. M1 and M2 had more sweet, metallic and liver flavour compared to A and AB. Sharp increases in the spoilage attributes (queasy, sour, and off flavour) was observed on day 13 with the sour flavour score increasing to above 20 (on the scale 0 – 100) in A and AB which is the acceptability limit in QDA (Magnússon *et al.* 2006; Odoli 2009).This indicates that the samples were approaching end of shelf life. There was no significance difference in flavour attributes between A and AB during storage, but M1 and M2 were significantly different ($p < 0.05$) on day 16 with respect to rancid and off flavour.

Texture attributes neither increased nor decreased consistently within the groups during storage. On day 6, sample group A had less meaty texture than AB, while no significance difference existed between the two groups with the other texture attributes (Appendix 2). The intensity of tender, juicy, and soft texture declined inconsistently up to day 10 in all the sample groups. Significance difference was observed in meaty and juicy texture attributes between air packed (A and AB) and MAP (M1 and M2) samples. However, M2 were tenderer than M1 on the same day. Apparently, texture attributes were not observed to be influenced by storage time. The variation in the intensity would have been as a result of gas atmosphere in the packages or sensitivity of the panellists to the attributes.

All samples showed no difference in flaky appearance during storage (Appendix 1). Significant difference in colour existed between A and AB on the initial day of the study with no consequential change observed until end of study. Difference between A and AB could have been a result of bleeding. Bleeding removes blood from the muscles thus improving the appearance of the fillets. In addition, bleeding reduces rancidity and off odour in fish. Bled fillets (AB) showed no off flavour until day 10 while the intensity of rancidity was low compared to non-bled sample (A) This can be correlated with the Torry score results that showed higher score in flavour and odour of the bled fillets up to day 10.Similar results on bled Asian sea bass slices were reported by Maqsood and Benjakul (2011).

5.4 Head space gas composition and drip loss measurements

In the preliminary study, the gas ratio was approximated as 2:1 in all the MAP packages therefore representing high possibility of interaction between the headspace gases and the packaged fillets. Under the superchilled conditions, the level of CO_2 declined 5 days post packaging in the MAP samples (Figure 4). It was higher in M3 (60 % CO2:40% Ar) and M4 (60% CO_2: 30% Ar: 10% N_2) MAP groups at 12.6% and 12.8% respectively compared to M1 (40% CO_2: 60% Ar) and M2 (40% CO_2: 60% N_2) groups at 11.0% and 10.9 % respectively. Decline in CO_2 composition was presumably due to its dissolution into the water phase of the fillet muscle (Masniyom 2011,Wang *et al.* 2008).

In the main study, CO_2 in the headspace declined consistently in the superchilled M1 (40% CO_2: 60% Ar) and M2 (40% CO_2: 60% NO_2) samples 6 days post packaging but the level remained

constant after day 6 in the chilled storage conditions (Figure 19). The results showed that superchilling increased absorption of CO_2 into the fish muscle while increase in temperature to 2°C reduced the process. This was in agreement with results by (Sivertsvik *et al.* 2003) that showed high dissolution of CO_2 in salmon fillets under superchilled storage compared to chilled storage. However, Wang *et al.* (2008) found no significant difference in CO_2 concentration of superchilled and chilled MAP cod loins due to formation of ice crystals that hindered absorption of the gas.

In both studies, drip loss was observed to increase with storage time in the MAP samples. High drip loss was observed in M3 (60 % CO_2:40% Ar) and M4 (60% CO_2: 30% Ar: 10% N_2) samples on day 11 compared to the other samples air (A), vacuum (V), M1 (40% CO_2: 60% Ar) and M2 (40% CO_2: 60% N_2) in the preliminary study (Table 8). In the main study drip loss in M1 and M2 progressively increased with storage (Figure 18). Previous studies have linked increase in drip loss with dissolution of CO_2 (Wang *et al.* 2008, Sivertsvik *et al.* 2003a). Dissolution of CO_2 is associated with increase in carbonic acid in the fish muscle. Low pH reduces water-holding capacity of the proteins leading to exudate loss from the fish muscle. High drip loss negatively affects the texture of the fillets and leads to nutrient loss. Studies have also shown that pre-treatment of fillets with additives such as sodium chloride solution and phosphates before packaging effectively reduced exudate loss (Pastoriza *et al.* 1998, Turan *et al.* 2003).

5.5 Microbiological and physical analysis

The initial microbial quality of the raw material in the main study varied when evaluated on the first day (Figure 16). Whole raw fish (bled and un-bled) were processed two days post catch. AB had high TVC count (log 3.3 CFU/g) compared to A (log 2.6 CFU/g). Both levels indicate acceptable quality (Pantazi *et al.* 2008, Kostaki *et al.* 2009). Similar observations were made by Sivertsvik *et al.* (2003) on initial level TVC (3.9 log CFU/g) during the shelf life study of super chilled cod fillets. However, higher initial TVC level in AB affected the quality of the bled fillets throughout storage. This is can be presumed to be as a result of delayed processing in the factory. Results from this study differ from those of Maqsood and Benjakul (2011) that showed lower TVC in the bled compared to the unbled Asian seabass fillets during storage.

Proliferation of microbes in the air packaged fillets (A and AB) was higher than in MA packed (M1 and M2) fillets under superchilled conditions (day 0 – day 6). Growth of TVC in M1 and M2 was almost constant during superchilled storage indicating delayed microbial growth (increased lag phase) in the MAP samples. Sharp increase in the TVC after day 6 was as result of increase in temperature (2^0 C) that favoured microbial growth in all the samples. No significance difference ($p>0.05$) was observed between M1 and M2 throughout the study. Likely explanation was that superchilling caused high dissolution of CO_2 into the fish muscle creating an acidic environment that presumably inhibited growth of the microbes. Similar observations were made by Wang *et al.* (2008) on super chilled MA packaged cod fillets. After day 6, higher temperature reduced absorption of CO_2 into the fillet muscle thus reducing bacteriostatic effect. TVC values for bled and unbled air packaged fillets (AB and A) exceeded 7 log CFU/g, the upper limit for fish to be safe for consumption (ICMSF 2002) on day 10 while M1 and M2, the level was attained six days later(around day 16. This is can be presumed to be a result of delayed processing in the factory. Results from this study differ from those of Maqsood and Benjakul (2011) that showed lower TVC in the bled compared to the unbled Asian seabass fillets during storage.

5.6 Comparison of quality evaluation methods (QIM, Torry, QDA, Microbial) and determination of shelf life

Based on freshness evaluation of raw and cooked fillets in the preliminary study, the sensory quality of M1 (40% CO_2: 60% Ar) and M2 (40% CO_2: 60% N_2) MAP samples was observed to be superior to counterpart air (A), vacuum (V), M3 (60% CO_2: 40% Ar) and M4 (60% CO_2: 30% Ar: 10% N_2) in superchilled storage. Extended storage life was found in the M1 and M2. On day 13 the MA packed samples had not reached sensory rejection while the other samples were rejected by 11^{th} day of storage. M3 and M4 were presumed to be affected by gas composition in the package. It was concluded that headspace gas in M1 and M2 samples had better influence on sensory quality of the fillets in superchilled storage and therefore recommended for the subsequent shelf life study.

In the shelf life study, average QI in all the sample groups (A, AB, M1 and M2) increased with storage time and a high correlation between the QI and storage time during storage was observed. Initial low QI corresponded to high Torry scores and QDA fresh attributes. According to Martinsdóttir *et al.* (2001), Torry score of 5.5 determines end of sensory shelf life (limit for consumption) due to prevalence of spoilage indicators. Sample A and AB reached the limit of consumption on day 11while it was extended in M1 and M2 to day 13 and 14 respectively. Torry score of 5.5 corresponded to QI score 7 for A and AB, 8 for M1 and 9 for M2 samples. In addition, this torry score corresponded to high microbial population beyond the acceptability limit (log 7.0 CFU/g) for A and AB while relatively lower levels were found in M1 and M2 at log 5.5 and 6.0 CFU/g respectively. Between day 10 and 13, spoilage related attributes flavour and odour attributes (cloth, TMA, sour, queasy) in QDA were highly detectable and scored above acceptable limit of 20 in A and B. End of shelf life is determined when average sensory score using QDA is above 20 (Cardenas Bonilla *et al.* 2007, Wang *et al.* 2008) on a scale of 0- 100. These results therefore revealed that, high proliferation of specific spoilage organisms in the TVC were responsible for spoilage and development of off odour and flavour in the fillets. However, the rate of proliferation was observed to vary in the sample groups due different atmospheric conditions in the packages. Lower TVC levels in the M1 and M2 corresponded to longer sensory shelf life compared to A and AB. Superchilling extends lag phase of microbial growth (Huss 1995). In this study, superchilling and MAP showed lag phase period in M1 and M2 between day 0 and day 6. This resulted to an increase in shelf life in MAP samples by 2- 3 days compared to the air packaged fillets (11 days). Similar findings were shown by Wang *et al.* (2008) and Sivertsvik *et al.* (2003).

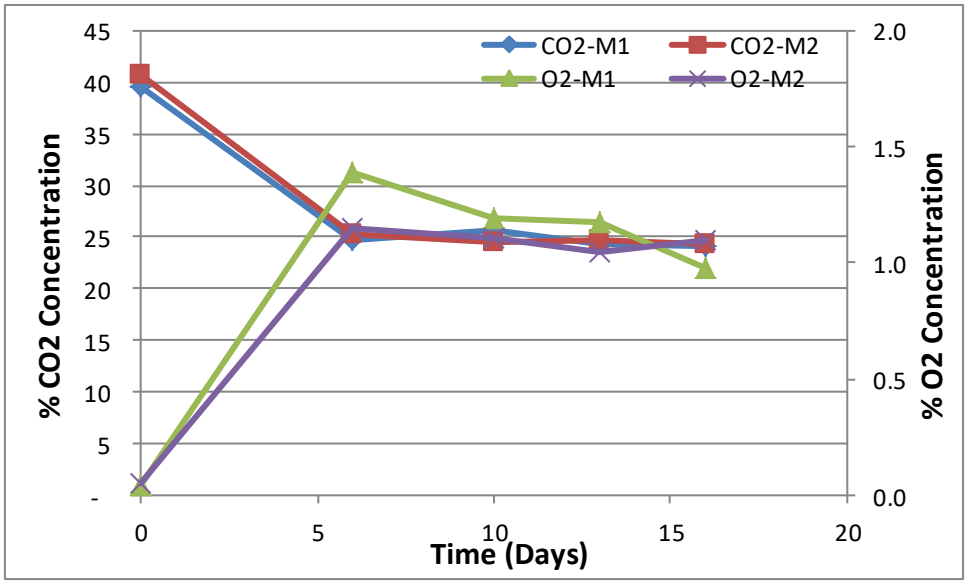


Figure 19: Mean concentration of headspace CO_2 and O_2 in the MAP packages (n=4) during storage.

6 CONCLUSIONS AND RECOMMENDATIONS

The preliminary study results showed the effect of different gas combinations in the package atmosphere on the sensory quality of A (air), V (vacuum), M1 (40% CO_2: 60% Ar), M2 (40% CO_2: 60% N_2), M3 (60% CO_2: 40% Ar) and M4 (60% CO_2: 30% Ar: 10% N_2) samples. Considering Torry score of 5.5 as the sensory rejection point, the shelf life of the A was determined to be 9 days and 11 days for A, M3 and M4 sample groups. M1 and M2 were observed to keep longer with to maximum of 13 days. Based on the quality characteristics observed throughout the preliminary study, gas combination in M1 and M2 were considered for MAP in the subsequent shelf life study.

QIM scheme developed for deskinned redfish fillets consisted of six quality attributes, which gave a total Quality Index of 16 points. Evaluation of the scheme showed linear correlation of QI for the samples (A, AB, M1 and M2) with storage time. The highest QI of 10 points was obtained in air packaged (A and AB) and MAP (M1 and M2) groups. These results recommend use of the QIM scheme to estimate the storage time of the sample groups in superchilled and chilled storage conditions. The scheme consisted of detailed descriptions of the attributes therefore a useful tool in training assessors. Evaluation of raw fillets using the scheme should be based on use of several assessors. The results on panellists' performance in this study emphasized the importance of training before evaluating raw fish using the scheme. Biological variation among the individual fillets was observed. This study therefore recommends use of at least 5 fillets per sample group in future studies.

Sensory analysis of cooked fillets and microbial analysis showed linear development of spoilage attributes with storage time. The Sensory evaluation with QIM was in agreement with results of the other methods, Torry, QDA and microbial analysis. The results of Torry, QDA and microbial

analysis were used to determine end of shelf life. When that information is available QIM can be used to estimate remaining shelf life. Considering the Torry and QDA results, sensory rejection occurred at day 11 for A and AB, and between day 14 for M1 and M2 groups. The results of this study showed extended storage time in the MA packaged groups by 3 days compared to the air packaged samples Bled and the non- bled fillets had the same shelf life however, bleeding was observed to improve the appearance and odour over the non-bled fillets.

ACKNOWLEDGEMENTS

I would like to show my gratitude to Dr. Tumi Tómasson, Programme Director of United Nations Fisheries Training Programme (UNU-FTP) for the training opportunity and further acknowledge UNU FTP for the training sponsorship.

I gratefully thank Mr. Þór H. Ásgeirsson, Ms.Sigríður Kr. Ingvarsdóttir and Mary Frances Davidson for their support and guidance while undertaking the programme.

I am heartily thankful to my supervisors, Kolbrún Sveinsdóttir and Emilia Martinsdóttir for their valuable advice and assistance in the project.

Special thanks to the Matis personnel, more particularly to the panel leader, Aðalheiður Ólafsdóttir and the team of panellists for their availability and support during the sensory evaluation sessions.

LIST OF REFERENCES

Ababuoch, L. (2010). Fish utilization and trade. In: *Second International Congress on Seafood Technology on Sustainable, Innovative and Healthy Seafood*. (eds J. Ryder, L. Ababouch and M. Balaban). Rome: FAO pp 9–31.

Anonymous (1996). *Council regulation (EC) No-2406/96 of 26 November 1996*.

Bao, H.N.D., Arason, S. and Porarinsdottir, K.A. (2007). Effects of Dry Ice and Superchilling on Quality and Shelf Life of Arctic Charr (Salvelinus alpinus) Fillets Effects of Dry Ice and Superchilling on Quality and Shelf Life of Arctic Charr (Salvelinus alpinus) Fillets ∗. *International Journal of Food Engineering* 3(3).

Bogdanović, T., Šimat, V., Frka-Roić, A. and Marković, K. (2012). Development and application of quality index method scheme in a shelf-life study of wild and fish farm affected bogue (Boops boops, L.). *Journal of food science* 77: S99–106.

Borderías, A.J. and Sánchez-Alonso, I. (2011). First processing steps and the quality of wild and farmed fish. *Journal of food science* 76: R1–5.

Cardenas Bonilla, A., Sveinsdottir, K. and Martinsdottir, E. (2007). Development of Quality Index Method (QIM) scheme for fresh cod (Gadus morhua) fillets and application in shelf life study. *Food Control* 18: 352–358.

Choubert, G., Brisbarre, F., Parfouru, D. and Baccaunaud, M. (2008). Argon Modified Atmosphere Packaging for Fillets of Rainbow Trout (Oncorhynchus mykiss) Fed Astaxanthin or Canthaxanthin. *Journal of Aquatic Food Product Technology* 17: 117–136.

Duun, A.. and Rustad, T. (2007). Quality changes during superchilled storage of cod (Gadus morhua) fillets. *Food Chemistry* 105: 1067–1075.

Food and Agricultutal Organization of the United Nations (2012). *World Fisheries and Aquaculture*. Rome: FAO

Fernández, K., Aspe, E. and Roeckel, M. (2009). Shelf-life extension on fillets of Atlantic Salmon (Salmo salar) using natural additives, superchilling and modified atmosphere packaging. *Food Control* 20: 1036–1042.

GOK FID (2007). The Fisheries (Safety of Fish, Fishery Products and Fish Feed) Regulations (Legal Notice No. 170/2007). Fisheries department, Kenya.

GOK FID (2008). Fisheries annual statistical bulletin. Fisheries department, Kenya.

GOK FID (2012). Marine frame survey. Fisheries department, Kenya.

Hansen, L., Gill, T., Røntved, S. and Huss, H. (1996). Importance of autolysis and microbiological activity on quality of cold-smoked salmon. *Food Research International* 29: 181–188.

Huss, H.H. (1995). Quality and quality changes in fresh fish. FAO Fisheries technical paper . No. 348, 195p pp. Rome: FAO.

Hyldig, G., Bremer, A., Martinsdottir, E. and Schelvis, R. (2007). Quality Index Methods. In: *Handbook of meat, poultry & seafood quality.* Blackwell Publishing, Oxford, UK, pp 529–49.

Hyldig, G. and Green-Petersen, D.M. (2004). QIM - an objective tool for determination of sensory quality. *Journal of Aquatic Food Product Technology* 13: 71– 80.

Icelandic Fisheries (2012). Fresh Processing methods and Products. Ministry of Fisheries and Agriculture, Iceland. [December 2012]. <http://www.fisheries.is/products/processing-methods/fresh/ >

ICMSF (2002). International Commission on Microbiological Specifications for Foods. Microorganisms in Food 7. Microbiological Testing in Food Safety Management. New York.

ISO (1994). 11035: Sensory analysis -- Identification and selection of descriptors for establishing a sensory profile by a multidimensional approach.

ISO (1993). Sensory Analysis. General Guidance for Selection, Training and Monitoring of Assessors. Part 1: Selected Assessors. Standard No. 8586–1,The International Organization for Standardization.

Kostaki, M., Giatrakou, V., Savvaidis, I.N. and Kontominas, M.G. (2009) Combined effect of MAP and thyme essential oil on the microbiological, chemical and sensory attributes of organically aquacultured sea bass (Dicentrarchus labrax) fillets. *Food microbiology* 26: 475–82.

Labbe, D., Rytz, A and Hugi, A. (2004). Training is a critical step to obtain reliable product profiles in a real food industry context. *Food Quality and Preference* 15: 341–348.

Lauzon, H., Sveinsdóttir, K. and Karlsdóttir, M. (2011). Effect of temperature control on the efficiency of modified atmosphere packaging of cod loins in bulk. Matis Report 21-11.

Love, R.M. (2001). Dark colour in white fish flesh. Torry Advisory Note, No. 76. [December 2012] <http://www.fao.org/wairdocs/tan/x5947E/x5947e00.htm>

Magnusson, H. and Martinsdottir, E. (1995). Storage quality of frozen and thawed fish in ice. *Journal of food science* 60: 273–278.

Magnússon, H., Sveinsdóttir, K., Lauzon, H.L., Thorkelsdóttir, A. and Martinsdóttir, E. (2006). Keeping quality of desalted cod fillets in consumer packs. *Journal of food science* 71(2): M69-76.

Magnússon, H., Sveinsdóttir, K., Þorvaldsson, L., *et al.* (2010). The effect of different cooling techniques on the quality changes and shelf life of whole cod (Gadus morhua). 28 pp. Reykavik, Iceland.

Maqsood, S. and Benjakul, S. (2011). Effect of bleeding on lipid oxidation and quality changes of Asian seabass (Lates calcarifer) muscle during iced storage. *Food Chemistry* 124: 459–467.

Martinsdottir, E. (2002). Quality management of stored fish. In: *Safety and quality issues in fish processing*. Woolhead Publishing, Reykavik, pp 360–378.

Martinsdóttir, E., Luten, J.B., Schelvis-Smit, A.A.. and Hyldig, G. (2003). Developments of QIM-past and future. In: *Quality Of Fish From Catch To Consumer: Labelling, Monitoring and Traceability*. (eds J.B. Luten, J. Oehlenschläger and G. Ólafsdóttir). Wageningen Academic publishers, Wageningen, Netherlands, pp 265– 272.

Martinsdottir, E., Schelvis, R., Hylding, G. and Sveindottir, K. (2009). Sensory evaluation of seafood:general princiles and guidelines. In: *Fishery Products: Quality, safety and authenticity*. (eds H. Rehbein and J. Oehlenschläger). Oxford: Wiley-Blackwell pp 411–443.

Martinsdóttir, E., Sveinsdóttir, K., Luten, J., Schelvis-Smit, R. and Hyl-dig, G. (2001). *QIM Eurofish- manual English version*. QIM Eurofish.

Masniyom, P. (2011). Deterioration and shelf-life extension of fish and fishery products by modified atmosphere packaging. *Songklanakarin J. Sci. Technol.* 33: 181–192.

Meilgaard, M., Civille, G.V. and Carr, B.T. (1991). *Sensory Evaluation Techniques*, Second Edi. Boca Raton: CRC Press.

Murray, J., Delahunty, C. and Baxter, I. (2001). Descriptive sensory analysis: past, present and future. *Food Research International* 34: 461–471.

Odoli, C.O. (2009). Optimal storage conditions for fresh farmed tilapia (*Oreochromis niloticus)* fillets. 92.

Ólafsdóttir, A., Margeirsson, B., Sveinsdóttir, K., Arason, S., Reynisson, E. and Martinsdóttir, E. (2012). Effect of superchilled processing of whole whitefish – pre rigor. 41 pp. Reykavik, Iceland.

Olafsdottir, G., Lauzon, H.L., Martinsdottir, E., Oehlenschlager, J. and Kristberg, K. (2006). Evaluation of Shelf Life of Superchilled Cod (Gadus morhua) Fillets and the Influence of Temperature Fluctuations During Storage on Microbial and Chemical Quality Indicators. *Journal of food science* 71: 97–109.

Olafsdottir, G., Martinsdottir, E., Oehlenschbger, J., *et al.* (1997). Methods to evaluate fish freshness in research and industry. *Trends in food science and technology* 8: 258–266(9).

Pantazi, D., Papavergou, A., Pournis, N., Kontominas, M.G. and Savvaidis, I.N. (2008). Shelf-life of chilled fresh Mediterranean swordfish (Xiphias gladius) stored under various packaging conditions: microbiological, biochemical and sensory attributes. *Food microbiology* 25: 136–43.

Pastoriza, L., Sampedro, G., Herrera, J.J. and Cabo, M.L. (1998). Influence of sodium chloride and modified atmosphere packaging on microbiological, chemical and sensorial properties in ice storage of slices of hake (Merluccius merluccius). *Food Chemistry* 61: 23–28.

Provincial, L., Gil, M., Guillén, E., Alonso, V., Roncalés, P. and Beltrán, J. a. (2010). Original article: Effect of modified atmosphere packaging using different CO_2 and N_2 combinations on physical, chemical, microbiological and sensory changes of fresh sea bass (Dicentrarchus labrax) fillets. *International Journal of Food Science & Technology* 45: 1828–1836.

QIM Eurofish (2012) The iPhone Application "How fresh is your fish?" based upon QIM. Available at: http://www.qim-eurofish.com.

Ravi Sankar, C.N., Lalitha, K. V, Jose, L., Manju, S. and Gopal, T.K.S. (2008). Effect of packaging atmosphere on the microbial attributes of pearlspot (Etroplus suratensis Bloch) stored at 0-2 degrees C. *Food microbiology* 25: 518–28.

Richards, M.P. and Hutlin, H.O. (2002). Contributions of Blood and Blood Components to Lipid Oxidation in Fish Muscle. *Journal of agricultural and food chemistry* 50: 555–564.

Rodríguez-Jérez, J.J., Hernández-Herrero, M.M. and Roig-Sagués, A.X. (2004). New methods to determine fish freshness in research and industry. 8.

Roth, B., Torrissen, O.J. and Slinde, E. (2005). The effect of slaughtering procedures on blood spotting in rainbow trout (Oncorhynchus mykiss) and Atlantic salmon (Salmo salar). *Aquaculture* 250: 796–803.

Sant'Ana, L.S., Soares, S. and Vaz-Pires, P. (2011). Development of a quality index method (QIM) sensory scheme and study of shelf-life of ice-stored blackspot seabream (Pagellus bogaraveo). *LWT - Food Science and Technology* 44: 2253–2259.

Shewan, J., Macintosh, R.G., Tucker, C.G. and Ehrenberg, A.S.C. (1953). The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. *Journal of Science Food Agriculture* 4: 16.

Sivertsvik, M., Jeksrud, W.K. and Rosnes, J.T. (2002). A review of modified atmosphere packaging of fish and fishery products – significance of microbial growth, activities and safety. *International Journal of Food Science & Technology* 37: 107–127.

Sivertsvik, M., Rosnes, J. and Kleiberg, G. (2003). Effect of modified atmosphere packaging and superchilled storage on the microbial and sensory quality of Atlantic salmon (Salmo salar) fillets. *Journal of food science* 68: 1467–1472.

Soccol, M.C.H. and Oetterer, M. (2003). Use of Modified Atmosphere in Seafood Preservation. *Brazilian Archives of Biology and Technology* 46: 569–580.

Stone, H. and Sidel, J.L. (2004). *Sensory Evaluation Practices*, Third Edit. Elsevier Academic Press, Redwood city, California,USA.

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

Tomic, O., Nilsen, A., Martens, M. and Næs, T. (2007). Visualization of sensory profiling data for performance monitoring. *LWT - Food Science and Technology* 40: 262–269.

Turan, H., Kaya, Y. and Erkoyuncu, Ä. (2003). Effects of glazing, packaging and phosphate treatments on drip loss in rainbow trout (Oncorhynchus mykiss W. 1792) during frozen storage. *Turk. J. Fish. Aquat. Sci* 109: 105–109.

Wang, T., Sveinsdóttir, K., Magnússon, H. and Martinsdóttir, E. (2008) Combined application of modified atmosphere packaging and superchilled storage to extend the shelf life of fresh cod (Gadus morhua) loins. *Journal of food science* 73: 79.

APPENDIX

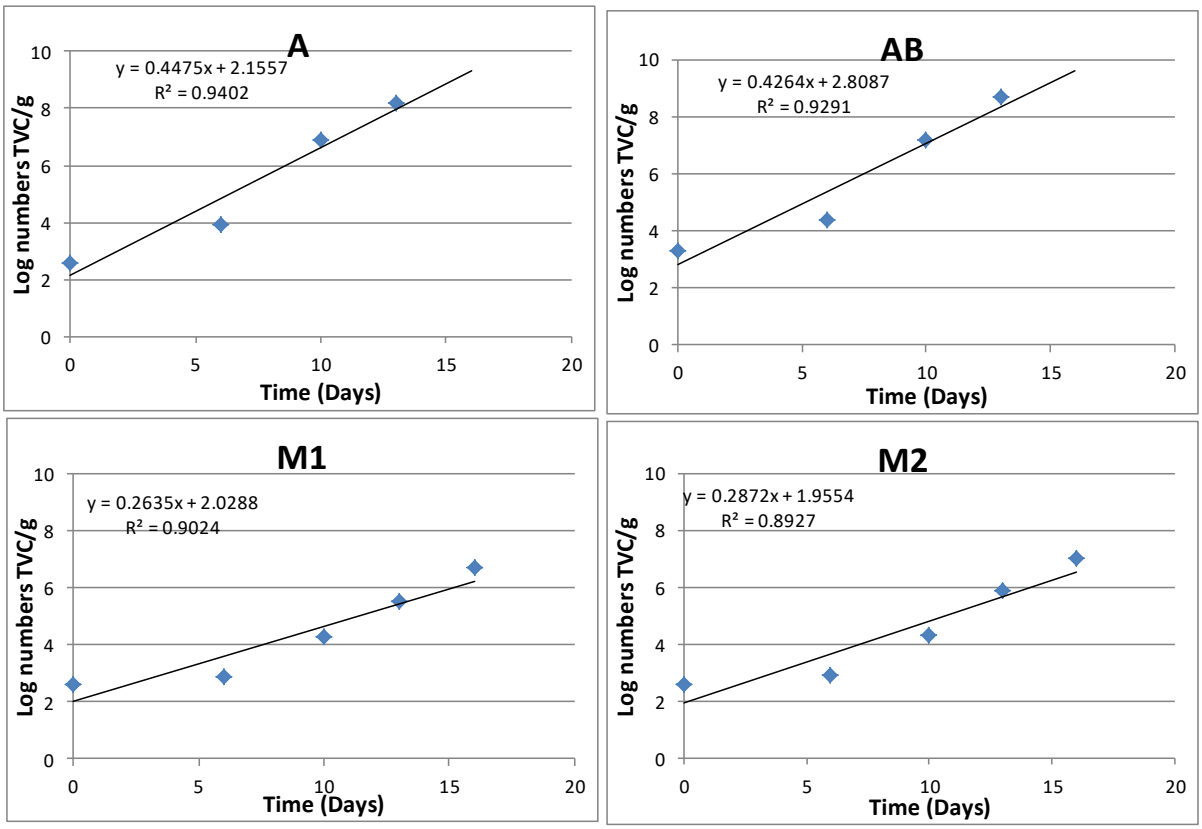
Appendix 1: Mean sensory scores of odour and appearance attributes of cooked redfish (average scores n = 2).

	Odour										Appearance		
Sample/ Time (d)	**Sweet**	**Liver**	**Shellfish**	**Vanilla**	**Rancid**	**Cloth**	**TMA**	**Queasy**	**Sour**	**Sulphur**	**Colour**	**Precipit.**	**Heterog.**
A-D0	40	29	17	7	1	1	0	1	1	0a	34a	21	29a
AB-D0	44	33	21	5	0	2	0	0	0	0b	21b	17	20b
p-value	0.4532	0.1933	0.2983	0.4284	0.0000	0.5435	1.0000	0.1727	0.2084	**0.0071**	**0.0132**	0.3323	**0.0169**
A-D6	31	22	13	8	2	7	2	4	1	0	36	32	33
AB-D6	38	25	16	11	1	4	1	1	1	0	30	27b	28
M1-D6	34	22	17	10	1	5	1	2	1	0	34	37a	34
M2-D6	34	20	16	9	1	6	1	2	1	1	32	39a	30
p-value	0.4274	0.4838	0.7494	0.5496	0.2566	0.3968	0.4166	0.2789	0.7675	0.9124	0.6480	**0.0174**	0.6245
A-D10	30	19	11	10	0	6	7a	2	9a	0	31	33	29
AB-D10	31	19	10	13	1	9	6a	6	4b	1a	28	31	25
M1-D10	37	23	12	13	1	8	2b	2	0c	0b	34	36	36
M2-D10	38	22	12	13	2	7	1b	3	1c	0b	38	35	30
p-value	0.2167	0.2311	0.5605	0.8592	0.2243	0.7475	**0.0028**	0.1407	**0.0000**	**0.0342**	0.1795	0.1796	0.0895
A-D13	9b	4b	3b	5b	6ab	25a	22a	20a	27a	11a	40	29b	37
AB-D13	11b	5b	4	4b	8a	28a	28a	23a	28a	13a	39	28b	30
M1-D13	20a	11a	7	9a	1c	12b	6b	2b	7b	1b	35	45a	37
M2-D13	26b	13a	8a	13a	1bc	8b	4b	2b	1b	0b	37	43a	37
p-value	**0.0000**	**0.0022**	**0.0235**	**0.0001**	**0.0127**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.6995	**0.0002**	0.2406
M1-D16	15	6	5	6b	5	24	27	8	13	5	46	38	40
M2-D16	14	6	6	11a	5	28	28	8	15	3	41	41	43
p-value	0.7681	0.7388	0.3551	**0.0234**	0.7134	0.2957	0.8525	0.7439	0.5366	0.2735	0.1791	0.3181	0.5132

Appendix 2: Mean sensory scores of flavour and texture attributes of cooked redfish (average scores n = 2).

	Flavour								Texture				
Sample/storage time	**Liver**	**Metallic**	**Sweet**	**Rancid**	**Queasy**	**Sour**	**TMA**	**Off**	**Soft**	**Juicy**	**Tender**	**Meaty**	**Sticky**
A-D0	30	33	40	1	1	0	0	0	49	50	57	16b	32
AB-D0	33	39	46	0	1	0	0	0	45	52	55	22a	26
p-value	0.2783	0.1441	0.0722	0.1664	0.8081	0.7661	0.3293	0.5701	0.4368	0.5796	0.6020	**0.0221**	0.0981
A-D6	29	27	28	4	3	1	3a	2	54	50	58a	10b	24
AB-D6	28	33a	32	1	2	1	1	0	47	42	50	18a	31
M1-D6	24	28	28	2	2	2	1	0	49	47	52	16a	30
M2-D6	27	23b	31	2	2	2	1b	0	45	44	45b	19a	30
p-value	0.4836	**0.0235**	0.6019	0.4105	0.9002	0.8394	0.0693	0.2820	0.0743	0.1574	**0.0076**	**0.0008**	0.2234
A-D10	24	30	27	0	3	0	4	2	47	44	50	12	30
AB-D10	23	27	28	1	4	1	3	0	42	41	48	13	29
M1-D10	23	30	28	1	2	0	2	0	41	40	45	10	34
M2-D10	24	34	31	2	4	1	4	0	45	44	44	12	30
p-value	0.9394	0.2500	0.4976	0.4888	0.7230	0.2668	0.7610	0.4723	0.3877	0.2593	0.6057	0.5304	0.6025
A-D13	7b	7b	7b	5	15a	21a	7a	16a	40	35b	43c	10b	20
AB-D13	5b	7b	7b	4	19a	19a	8a	16a	37b	39b	43c	10b	18b
M1-D13	20a	18a	24a	0	3b	7b	5	4b	45	46a	50b	19a	23
M2-D13	22a	22a	26a	1	5b	0b	1b	0b	49a	47a	55a	19a	27a
p-value	**0.0000**	**0.0007**	**0.0000**	0.2441	**0.0001**	**0.0000**	**0.0262**	**0.0000**	**0.0192**	**0.0004**	**0.0000**	**0.0051**	**0.0420**
M1-D16	14	10	20	10a	13	6	11	3b	53	40	48	18	33a
M2-D16	16	10	15	3b	10	10	11	9a	49	35	43	16	21b
p-value	0.6769	0.5930	0.2358	**0.0025**	0.4469	0.3439	0.9031	**0.0064**	0.5995	0.4339	0.6109	0.5668	**0.0070**

Appendix 3: Linear relationship between TVC and storage time in all sample groups (A, AB, M1 and M2).



Appendix 4: Torry Freshness score sheet for cooked iced redfish.

score	Odour	Flavour
10	Initially weak odour of boiled cod liver, fresh oil, starchy	boiled cod liver Watery, metallic.
9	Shellfish, seaweed, boiled meat, oil, cod liver	oily, boiled cod liver Sweet, meaty characteristic.
8	Loss of odour, neutral odour	Sweet and characteristic flavours but reduced in intensity.
7	Wood shavings, wood sap, vanillin	Neutral
6	Condensed milk, boiled potato	Insipid
5	Milk jug odours boiled clothes- like	Slight sourness, trace of "off"-flavours, rancid
4	Lactic acid, sour milk TMA	Slight bitterness, sour, "off"-flavours, TMA rancid
3	Lower fatty acids (e.g. acetic or butyric acids) composed grass, soapy, turnipy, tallowy	Strong bitter, rubber, slight sulphide rancid